

Physiological Ecology and Vulnerability to Climate Warming in *Anolis*.

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
Biology in the Graduate School
of Duke University

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ABSTRACT

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Abstract

Human activity has resulted in significant increases in air temperature over the last century, and air temperatures are expected to continue rising at an accelerating rate over the next 100 years (IPCC 2007). The warming that has already occurred has had significant impacts on the world's biota: species ranges are shifting north (or upslope), seasonal phenological events are occurring earlier, disease dynamics are changing, and populations are going extinct (Walther *et al.* 2002; Parmesan & Yohe 2003; Parmesan 2006; Walther 2010; Pau *et al.* 2011). Understanding the temperature-dependent biological mechanisms that lead to such changes is a major priority: only with such understanding can we hope to make a concerted effort to mitigate the effects of continuing climatic change.

There are three general biological mechanisms by which organisms can respond to, and potentially buffer themselves from, the direct effects of climate change: 1) physiological plasticity, 2) behavior, and 3) evolution. Here, I refer to physiological plasticity as changes in thermal reaction norms, which include sensitivity to thermal change and tolerance for thermal extremes (Huey & Stevenson 1979). These plastic responses can be reversible (acclimation) or be fixed by developmental or cross-generational non-genetic processes (West-Eberhard 2003; Ghalambor *et al.* 2007; Angilletta 2009). There appear to be global patterns of plasticity in thermal physiology,

as temperate ectotherms tend to be more plastic than tropical ectotherms (Feder 1978; Tsuji 1988; Ghalambor *et al.* 2006). This difference is hypothesized to result from differences in seasonality: temperate ectotherms can experience a much wider range of thermal conditions than tropical ectotherms, and thus temperate environment might select for plasticity to track changing conditions. If physiological plasticity can buffer organisms from warming (Stillman 2003; Somero 2010), then tropical ectotherms may be at a disadvantage in the face of climate change (Huey *et al.* 2009).

In general, behavioral responses to thermal challenges can be thought of as occurring on either local or regional scales. At the local scale ectotherms can engage in behavioral thermoregulation, seeking out thermally suitable microhabitats within their home ranges (Bogert 1949; Huey *et al.* 2003; Kearney *et al.* 2009). At the regional scale, organisms may shift their ranges by migrating along elevation or latitudinal thermal gradients (usually up or north, respectively) to escape warming (Buckley *et al.* 2013). The degree to which local- and regional-scale behavioral responses can buffer populations from warming depends on numerous factors. For example, behavioral thermoregulation requires fine-scale thermal variation within the environment. However, habitats such as heavily shaded tropical forests have little thermal heterogeneity, precluding behavioral thermoregulation as an effective buffering mechanism (Huey *et al.* 2009). On the other hand, range shifts can be hindered by factors such as inherent mobility and natural and man-made barriers (Forero-Medina *et al.* 2011). Both behavioral thermoregulation and

migration can be hindered by the presence of competitors or antagonistic species such as predators or parasites in thermally favorable locations (Araújo & Luoto 2007).

Most work on the evolution of thermal physiology focuses on the evolution of thermal tolerance limits (i.e., the lower and upper lethal temperature thresholds) (Stillman & Somero 2000; Angilletta *et al.* 2007; Barrett *et al.* 2011). Broad-scale comparative analyses have demonstrated that upper thermal limits vary less than lower thermal limits, suggesting that upper thermal limits may be evolutionarily constrained (Kellermann *et al.* 2012; Araújo *et al.* 2013; Grigg & Buckley 2013). This pattern is particularly strong looking over terrestrial latitudinal gradients; cold tolerance increases with latitude, but heat tolerance does not change appreciably (Sunday *et al.* 2012). Artificial selection experiments have demonstrated that the upper thermal tolerances of animals can evolve, but there may be limits to how much they can change (Huey *et al.* 1991; Loeschcke & Krebs 1996).

Physiology, behavior, and evolution are of course not mutually exclusive mechanisms. As noted above, physiological traits such as tolerance to extreme temperature can evolve, as can behavioral mechanisms. In addition, behavior may promote or inhibit the evolution of physiology. For example, behavioral thermoregulation can potentially inhibit the evolution of thermal physiology because it allows organisms to buffer themselves from thermal change (Huey *et al.* 2003). Furthermore, the physiological state of an organism can dictate how it behaviorally

responds to a given stimulus (Atkins-Regan 2005). For example, lizards that are dehydrated seek out cooler microclimates (Crowley 1987).

My dissertation focuses on the physiological, behavioral, and evolutionary axes of organismal response to climatic challenges, and their interactions, using the arboreal Caribbean lizard *Anolis cristatellus* as a model system. The general approach that I take throughout each chapter is to consider climatic data and organismal responses to climate at a fine-scale. A recent review and meta-analysis of climate change studies found that, on average, researchers consider climatic data at a scale 10,000X larger than the animals they study (Potter *et al.* 2013). In other words, we frequently consider the climatic environment very coarsely relative to our focal organisms. Such an approach can yield broad patterns of warming vulnerability over large geographic scales. Nonetheless, much of the climatic variation important to organisms occurs at the scale of meters rather than kilometers (Helmuth *et al.* 2010). Similarly, broad-scale studies must typically make assumptions about how organisms respond to climatic variation, rather than actually measuring responses. Throughout, I highlight the benefits of working at the scale of the organism.

Chapter 1 is the only chapter that does not deal directly with thermal biology. In it, I investigate whether or not *A. cristatellus* from mesic and xeric habitats differ in their water loss rates, and ask whether the differences that I observe can be explained by plasticity (Gunderson *et al.* 2011). In the second chapter, I explore the vulnerability of *A.*

crisatellus to climate warming by integrating behavior and physiology with fine-scale measurements of the thermal environment (Gunderson & Leal 2012). In the third chapter, I investigate the ability of thermal tolerance limits to evolve rapidly in response to climatic change using the recent introduction of *A. crisatellus* to Miami from Puerto Rico (Leal & Gunderson 2012). In the final chapter, I focus solely on behavior and use *A. crisatellus* to ask how well current models of thermal constraint on activity predict observed patterns at a fine scale in the field (Gunderson and Leal, *in review*).

Dedication

For Katie. I can't imagine having done this without you.

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Acknowledgements

I would like to thank the members of my dissertation committee for all of their help over the years. I would especially like to thank my advisor, Manuel Leal: I cannot imagine a better mentor. Members of the Leal Lab have been great colleagues and have greatly assisted all aspects of my work, from the field to the laboratory to writing, especially B.J. Powell and D.S. Steinberg. The field components of my work could not have been done without the invaluable assistance of the Departamento de Recursos Naturales y Ambientales of Puerto Rico which provided all the necessary permits to complete this work, Paul Hertz for providing copper lizard models, CDR Daniel Kalal and the United States Navy for permission to work within the Roosevelt Roads Naval Base, El Verde Biological Station (University of Puerto Rico, Río Piedras), Mata de Plátano Field Station (Interamerican University, Bayamón), Isla Magüeyes Field Station (University of Puerto Rico, Mayagüez), the Jobos Bay Marine Reserve (U.S. National Oceanic and Atmospheric Administration), and Tony and Joan at T.J. Ranch. Field assistants included Kathleen Ferris, Neil Bacon, Jeremy Siegel, and Kristín Ocasio. Richard Nappi in the Physics machine shop helped me build numerous devices that I used throughout my work. I would also especially like to acknowledge the Biology Department Staff, including Anne Lacey and Jim Tunney, who went out of their way to make my life as a graduate student much easier. Funding was provided by grants from the National Science Foundation, including a Doctoral Dissertation Improvement Grant,

the Duke Center for Latin American and Caribbean Studies, the Duke chapter of Sigma Xi, the Duke Biology Department, the North Carolina Academy of Sciences, the Society of Integrative and Comparative Biologists, and the Animal Behavior Society.

1. Tests of the contribution of acclimation to geographic variation in water loss rates of the West Indian lizard *Anolis cristatellus*¹

1.1 Summary

Phenotypic plasticity can contribute to the process of adaptive radiation by facilitating population persistence in novel environments. West Indian *Anolis* lizards provide a classic example of an adaptive radiation, in which divergence has occurred along two primary ecological axes: structural microhabitat and climate. Adaptive plasticity in limb morphology is hypothesized to have facilitated divergence along the structural niche axis in *Anolis*, but very little work has explored plasticity in physiological traits. Here, we experimentally ask whether Puerto Rican *Anolis cristatellus* from mesic and xeric habitats differ in desiccation rates, and whether these lizards exhibit an acclimation response to changes in relative humidity. We first present microclimatic data collected at lizard perch sites that demonstrate that abiotic conditions experienced by lizards differ between mesic and xeric habitat types. In Experiment 1, we measured desiccation rates of lizards from both habitats maintained under identical laboratory conditions. This experiment demonstrated that desiccation rates differ between populations; xeric lizards lose water more slowly than mesic lizards. In Experiment 2, lizards from each habitat were either maintained under the conditions of

¹ This chapter has been published in the Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology.

Experiment 1, or under extremely low relative humidity. Desiccation rates did not differ between lizards from the same habitat maintained under different treatments and xeric lizards maintained lower desiccation rates than mesic lizards within each treatment. Our results demonstrate that *A. cristatellus* does not exhibit an acclimation response to abrupt changes of hydric conditions, and suggest that tropical *Anolis* lizards might be unable to exhibit physiological plasticity in desiccation rates in response to varying climatic conditions.

1.2 Introduction

Phenotypes can vary in response to the environmental conditions individuals experience during their lifetime, a phenomenon known as phenotypic plasticity (reviewed in West-Eberhard 2003). The expression of phenotypic plasticity (or a lack thereof) can have important ecological and evolutionary implications for populations, including influences on the interactions between species (Agrawal 2001), the evolution of phenotypic traits (Ghalambor *et al.* 2007), and the probability that species persist in the face of anthropogenic climate change (Stillman 2003). In some cases, plasticity may be mal-adaptive or neutral (DeWitt *et al.* 1998; Ghalambor *et al.* 2007). In other cases, plasticity appears to be adaptive in that the phenotype expressed in a particular environment is predicted to increase fitness relative to the phenotypes expressed in other environments. For example, the maximum critical lethal temperature of many fish

increases after exposure to warm temperatures, a change that should reduce the probability of mortality under natural conditions (Reynolds & Casterlin 1979).

Phenotypic plasticity could play an important role in the process of adaptive radiation by facilitating the persistence of populations that encounter new environmental challenges (West-Eberhard 2003; Sol *et al.* 2005; Pfennig & Pfennig 2009), a scenario that has been hypothesized for lizards in the genus *Anolis* (Losos *et al.* 2000). *Anolis* lizards have adaptively radiated on the Greater Antillean islands along two major ecological axes: structural niche and climatic niche (Williams 1972, 1983). Adaptive divergence in a suite of morphological traits, including limb size, is correlated with the occupation of different structural niches (e.g., tree trunks, twigs, grasses)(Losos 2009). Experimental manipulations with *A. carolinensis* and *A. sagrei* have demonstrated that limb size can change in response to the structural habitat (i.e., the distribution of available perch diameters) under which juveniles develop (Losos *et al.* 2000; Kolbe & Losos 2005). This developmental plasticity appears adaptive as it results in limbs predicted to be beneficial on the perch types used by influencing speed and stability (Losos *et al.* 2000; Spezzano & Jayne 2004; Losos 2009).

The other main axis of *Anolis* divergence is the climatic niche axis (Williams 1972; Losos 2009). The islands of the Greater Antilles are topographically complex and exhibit extensive variation in thermal conditions and rainfall, including high elevation rain forests and low elevation desert scrublands. Most species of anole occupy a distinct

climatic niche within each island (Ruibal 1961; Rand 1964; Williams 1972; Huey & Webster 1976; Hertz 1992a; Hertz 1992b), and physiological differences exist between species inhabiting different niches. For example, species found in warm, dry regions can tolerate higher temperatures and have lower rates of water loss than species from cool moist regions (Sexton & Heatwole 1968; Huey & Webster 1976; Hillman & Gorman 1977; Hertz *et al.* 1979; Hillman *et al.* 1979; Hertz 1980; Dmi'el *et al.* 1997; Perry *et al.* 1999, 2000). Historically, these differences had been interpreted as having a genetic basis. However, the degree of plasticity that can be exhibited by physiological traits has been little explored in anoles. For example, laboratory tests of acclimation capacity of cutaneous water loss rate, a trait thought to be important for maintaining water balance, have been conducted under controlled climatic conditions on only one species of anole, *A. carolinensis* (Kobayashi *et al.* 1983; Kattan & Lillywhite 1989). In this species, individuals significantly reduce their rates of cutaneous water loss after only 1 week of exposure to low-humidity conditions (Kobayashi *et al.* 1983; Kattan & Lillywhite 1989). However, whether or not the acclimation response observed in *A. carolinensis*, which is the only member of the West Indian radiation with an exclusively temperate distribution (Losos 2009), occurs in other anoles remains an open question.

Here, we evaluate geographic variation and phenotypic plasticity in water loss rates in the Puerto Rican *A. cristatellus*. With respect to phenotypic plasticity, we explicitly test the ability of adult lizards to acclimate to changes in relative humidity.

Previous research on water loss rates of *A. cristatellus* on the British Virgin Islands has demonstrated that populations from arid regions tend to have lower rates of water loss than those from wetter regions (Dmi'el *et al.* 1997; Perry *et al.* 1999, 2000). The authors suggested that this variation was at least partially due to phenotypic plasticity, although in a common garden experiment using outdoor enclosures they found no change in water loss rates of lizards from different regions over a 1-month period (Perry *et al.* 2000). Thus, it is unclear if water loss rates of *A. cristatellus* can exhibit an acclimation response to changing climatic conditions. Furthermore, rigorous tests for acclimation of water loss rates under controlled climatic conditions are currently lacking for a tropical anole.

In this study, we conducted experiments under controlled laboratory conditions to address the following questions. First, do populations of *A. cristatellus* inhabiting distinct climatic conditions on the island of Puerto Rico differ in water loss rates? Second, can individuals of *A. cristatellus* adjust their water loss rates in response to changes in humidity regimes? To achieve these goals we measured water loss rates on two populations of *A. cristatellus* inhabiting distinct habitat types (Rand 1964; Heatwole 1976): warm xeric forest with low rainfall and cooler mesic forest with relatively high rainfall (Ewel & Whitmore 1973; Daly *et al.* 2003). Since water loss rates of individuals in the field are dictated by the conditions of their immediate surrounding, and not by average climatic conditions across a large geographic range (Hertz 1980), we first

present data on the environmental conditions (air temperature, wind speed, relative humidity) experienced by lizards at their perch sites in the field. Next, we present data from experiments testing whether the water loss rates of individuals are correlated with habitat type. We predict that individuals from a xeric habitat should lose water at a lower rate than individuals from a mesic habitat. Finally, we evaluate if lizards are able to acclimate to changes in relative humidity. To do so we conducted a controlled experimental manipulation in which individuals were exposed to two distinct humidity regimes, while keeping the temperature constant across treatments. In particular, we ask whether or not mesic lizards converge on water loss rates of xeric lizards when individuals from both habitat types are housed under low-humidity conditions.

1.3 Methods

1.3.1 Study species and locations

Anolis cristatellus is a small-to-medium sized (maximum snout-vent length (SVL) for males 76mm, females 56 mm) arboreal lizard typically found on the ground or on tree trunks up to 2 m in height (a member of the “trunk-ground” ecomorph category, Williams 1983). *A. cristatellus* is endemic to the islands of the greater Puerto Rican Bank. On the island of Puerto Rico *A. cristatellus* can be found from sea level to mid-elevation (1,000 m) inhabiting mesic and xeric habitats (Rand 1964; Heatwole 1976).

We collected adult males and females of *A. cristatellus* and sampled habitat conditions at Cambalache State Forest (18°26' 35" N; 66°35' 47" W), a mesic forest

located in the karst region in the northwestern part of the island, and at Guánica State Forest (17°5801900N; 66°5200600W), a xeric forest on the southern coast. Cambalache State Forest can be characterized as a woodland with a mostly closed canopy and mean annual rainfall of 1,500 mm. Guánica State Forest is a xeric desert scrubland that receives only 760 mm of annual rainfall (Helmer *et al.* 2002). In addition, operative temperatures and lizard body temperatures are more than 3°C higher in Guánica than Cambalache during both the wet and dry season (see Chapter 2).

1.3.2 Perch microclimates

We measured air temperature, wind speed, and relative humidity at perch sites occupied by lizards from 14 to 30, July 2010. Data were collected at the perch sites of 176 individuals at the mesic site and 173 individuals at the xeric site. In each forest we slowly walked through the habitat from 0745 to 1715 hours, a period when lizards are active, looking for lizards. When a lizard was found, we collected data on the sex, perch height, lizard orientation, basking status, and weather conditions before approaching the lizard. At the perch site, air speed, air temperature, and relative humidity were measured with an anemometer/hygrometer (Omega, Inc., model HHF11) that was carried with its sensor exposed to decrease equilibration time. Air speed was taken over a period of approximately 10 s with the probe held perpendicular to the wind direction approximately 10 cm from the perch. The probe was then allowed to equilibrate for

approximately 2 min, at which time air temperature and relative humidity were recorded.

1.3.3 Experiment 1

We collected adult individuals at each site from August 22 to 23, 2009, and brought them back to our laboratory at Duke University. Lizards were housed individually in 299x219x21cm (L9W9H) plastic cages with a wooden dowel provided as a perch. Cages were kept in a climate-controlled growth chamber (Environmental Growth Chambers, model M25-3400) set at 28°C with a relative humidity (RH) of 60% (water vapor deficit, 1.50 kPa) and a 12L:12D light cycle with full spectrum light provided during daylight hours. All cages had mesh tops to facilitate the equilibration of cage air temperature and relative humidity with the growth chamber. Lizards were fed crickets supplemented with calcium powder three times weekly and watered twice daily during weekdays and once per day on weekends.

After approximately 3 months in captivity, we measured water loss rates by quantifying the change in body mass of lizards placed in an incubator (Percival, Inc., model I30NLC8) set at a constant temperature and humidity (following similar methodologies in Hillman & Gorman 1977; Hertz *et al.* 1979; Hertz 1980; Kobayashi *et al.* 1983; Kattan & Lillywhite 1989; Perry *et al.* 2000). Data were collected from 26 individuals (15 males and 11 females) in the mesic habitat and from 24 individuals (15 males and 9 females) in the xeric habitat. Before placing the lizards inside the incubator,

individuals were housed individually in modified 414 ml Tupperware containers with mesh tops and bottoms to allow for airflow. The day before the experiment, all containers were placed inside the incubator to dry them. On the day of the experiment, the mass of each container was measured immediately before and after a lizard was placed in it to calculate initial lizard mass. Immediately after taking these measurements, we placed containers with lizards on racks inside the incubator. The incubator was set at 30°C with a RH of 10% (RH ranged from 8 to 11% during the experiments). The 30°C temperature is within the preferred temperature range of *A. cristatellus* from both mesic and xeric habitats (Hertz *et al.* 1993), and falls within the range of temperatures experienced by *A. cristatellus* at each site (see Chapter 2, Gunderson & Leal 2012). The interior of the incubator was kept in total darkness, which, combined with the relatively small volume of the containers, should have drastically reduced if not eliminated lizard activity during the experiment. We weighed the containers with lizards every 2 h over an 8 h period. Mass was taken with an electric balance sensitive to 0.0001 g (Ohaus Inc., Adventurer Pro). If a lizard defecated during a particular 2-h period, we excluded the mass lost from that period in our calculations of water loss.

We calculated a number of metrics of water loss commonly used to compare differences in water loss rates among populations and species: mass-specific water loss (mg/g/h), percent body weight lost (BW%/h), area-specific water loss (mg/cm²/h), and

cutaneous resistance (R , s/cm). To calculate area-specific water loss, we calculated the surface area of each lizard using the following equation: $A = 1.16 \times 10^{-4} BW^{0.53}$ where BW is body weight in grams. This conversion was empirically derived specifically for *A. cristatellus* (Dmi'el *et al.* 1997). We assumed that 75% of the water loss we recorded occurred across the integument, as this has also been empirically determined for *A. cristatellus* at 30°C (Dmi'el *et al.* 1997). Similar values of percent cutaneous water loss have been shown for other lizards (Bentley & Schmidt-Nielsen 1966; Eynan & Dmi'el 1993). Skin resistance to water loss was calculated following Lillywhite and Sanmartino (1993).

1.3.4 Experiment 2

We conducted a second experiment to determine whether exposure to extremely low humidity would induce acclimation of desiccation resistance. We randomly divided the lizards used in the first experiment into two groups. One group continued to be maintained under the standard laboratory conditions described above (60% RH, 30°C, 12L:12D), whereas the other group was placed in a second climate-controlled room, in which all of the abiotic conditions were the same as in the other group except that RH was maintained at 30% (water vapor deficit, 2.63 kPa). Feeding and watering continued in both groups as described in Experiment 1. Lizards were maintained under these conditions for 2 weeks, at which time water loss rates of all individuals were estimated using the methodology described in Experiment 1. The congeneric *A. carolinensis* shows

an acclimation response after experiencing low-humidity conditions for only 1 week (Kobayashi *et al.* 1983; Kattan & Lillywhite 1989).

1.3.5 Statistical analyses

We tested our hypotheses using data on area-specific rates of water loss. We also present other metrics of water loss for comparison with other studies (Table 2). Mass-specific water loss correlates negatively with body size, most likely due to the differential scaling of mass and surface area (Hertz 1980). Xeric lizards were larger than mesic lizards (Table 2), and, thus, mass-specific metrics are biased towards detecting a difference between lizards from the two habitats based on size alone.

The data were not normally distributed and none of the transformations we used produced a normally distributed data set. Therefore, after confirming homogeneity of variances among groups (F tests, all $P > 0.050$), we used non-parametric Wilcoxon rank-sum tests on untransformed data for all comparisons. All statistical analyses were done using the R statistical programming package (R Development Core Team 2012).

1.4 Results

1.4.1 Perch microclimates

The microclimate conditions present at the lizards perch sites differ between habitat types. On average, lizards in the xeric habitat experienced air temperatures 2.2°C warmer than lizards in the mesic habitat ($P < 0.001$) (Table 1). In addition, relative humidity at xeric perch sites averaged 12.2% lower than at mesic perch sites ($P < 0.001$)

(Table1). Similarly, wind speed was significantly higher in the xeric habitat, by an average of 0.2 m/s ($P = 0.001$) (Table 1).

Table 1. Summary statistics of environmental data (mean \pm SE) collected at the perch sites of lizards in each habitat type. Data are partitioned by sex and weather conditions at the time of sampling. N sample size, T_{air} air temperature ($^{\circ}\text{C}$), wind speed (m/s), RH relative humidity.

	<u>N</u>	<u>T_{air}</u>	<u>Wind speed</u>	<u>RH</u>
Mesic habitat				
Female/cloudy	7	26.4 (0.1)	0.1 (0.1)	84.9 (0.9)
Female/sunny	115	28.0 (0.1)	0.6 (0.0)	83.7 (0.6)
Male/cloudy	5	26.7 (0.1)	0.0 (0.0)	86.6 (0.9)
Male/sunny	49	28.3 (0.2)	0.7 (0.1)	81.2 (0.9)
Total	176	28.0 (0.1)	0.6 (0.0)	83.1 (0.5)
Xeric habitat				
Female/cloudy	41	29.3 (0.2)	0.6 (0.1)	72.9 (1.0)
Female/sunny	71	30.8 (0.2)	0.8 (0.0)	69.1 (0.8)
Male/cloudy	28	29.7 (0.3)	0.7 (0.1)	73.3 (1.1)
Male/sunny	33	30.4 (0.4)	0.9 (0.1)	70.1 (1.5)
Total	173	30.2 (0.1)	0.8 (0.0)	70.9 (0.5)

1.4.2 Experiment 1

Among individuals from the same habitat type there were no significant differences in area-specific water loss rates between males and females (xeric lizards, $P = 0.290$; mesic lizards, $P = 0.217$) (Table 2). Thus, data from males and females are combined for all subsequent analyses. Lizards from the xeric habitat lost water at a significantly lower rate than individuals from the mesic habitat ($P = 0.003$) (Table 2),

congruent with the general trend observed in anoles in which individuals from xeric habitats are more resistant to desiccation.

Table 2. Summary statistics (mean \pm SE) for water loss rates of mesic and xeric individuals of *A. cristatellus* maintained under identical conditions of temperature and humidity ("Experiment 1"). MSWL = mass-specific water loss rate (mg/g/h), %BW/h = rate of water loss expressed as a percentage of body weight (BW), ASWL = area-specific water loss rate (mg/cm²/h), R = resistance (skin + boundary layer) of lizards to water loss (s/cm), Survival time = the estimated time, in hours, that an average individual would survive before reaching a lethal dehydration state under experimental conditions.

	<u>Sex</u>	<u>N</u>	<u>Lizard</u>					<u>Survival</u>
			<u>mass (g)</u>	<u>MSWL</u>	<u>% BW/h</u>	<u>ASWL</u>	<u>R</u>	<u>Time (h)</u>
Mesic	M	15	5.7 (0.1)	1.66 (0.08)	0.13 (0.01)	0.18 (0.01)	579 (27)	199
	F	11	1.9 (0.1)	2.62 (0.18)	0.20 (0.02)	0.16 (0.01)	651 (45)	125
	Both	26	-	-	-	0.17 (0.01)	609 (25)	-
Xeric	M	15	6.4 (0.2)	1.31 (0.07)	0.17 (0.01)	0.14 (0.01)	734 (44)	251
	F	9	2.2 (0.1)	2.00 (0.07)	0.26 (0.02)	0.13 (0.01)	805 (69)	147
	Both	24	-	-	-	0.14 (0.01)	760 (37)	-

1.4.3 Experiment 2

Area-specific water loss rates of lizards from the same habitat type did not differ between individuals placed at 30% RH and those at 60% RH (mesic lizards, $P = 0.851$; xeric lizards, $P = 0.426$) (Table 3), demonstrating the lack of an acclimation response to changes in hydric conditions over a 2-week period. In accordance with the results from Experiment 1, water loss rates of xeric lizards were lower than those of mesic lizards in both treatments (30% RH, $P = 0.058$; 60% RH, $P = 0.008$) (Table 3). Population estimates

of water loss rates increased between Experiment 1 and Experiment 2, irrespective of treatment or habitat of origin (Tables 2, 3).

Table 3. Summary statistics (mean \pm SE) for water loss rates of mesic and xeric individuals of *A. cristatellus* maintained under different drying regimes (“Experiment 2”). ASWL = area-specific rates of water loss (mg/cm²/h), R = resistance (skin + boundary layer) of lizards to water loss (s/cm).

		<u>N</u>	<u>ASWL</u>	<u>R</u>
Mesic	60% RH	10	0.28 (0.02)	367 (24)
	30% RH	14	0.27 (0.02)	385 (28)
Xeric	60% RH	10	0.20 (0.02)	530 (50)
	30% RH	11	0.22 (0.02)	467 (28)

1.5 Discussion

Our findings are consistent with the general consensus that species or populations living under distinct desiccation regimes exhibit different physiological capacities to resist water loss (Lillywhite 2006). The microhabitat data demonstrate that the perch sites occupied by mesic and xeric lizards differ significantly in abiotic conditions that influence desiccation rates. Air temperatures and wind speed were highest and relative humidity was lowest at xeric perch sites (Table 1). In addition, xeric lizards have higher body temperatures than mesic lizards (see Chapter 2). Theoretically, this confluence of factors should cause xeric lizards to experience a stronger evaporative

force driving water from their bodies than mesic lizards (Hertz 1980; Eynan & Dmi'el 1993).

As predicted, given the microclimate measurements and the general abiotic characteristics of each habitat type, we found that individuals of *A. cristatellus* from the dry xeric habitat lost water more slowly than lizards from the moist mesic habitat (Table 2). This difference was present even though lizards from both populations were maintained under identical conditions (i.e., 28°C, 60% RH) for over 3 months before the experiment. By keeping the lizards under the same conditions for such a long period, differences in water loss rates that resulted from ecological conditions experienced before being captured should be minimized, if not completely removed. Moreover, the difference between mesic and xeric lizards was maintained irrespective of the conditions under which the lizards were maintained over a 2-week period (Table 3). This finding supports a lack of a physiological acclimatory response to changes in desiccation regimes. It has also been shown that metabolic rate does not acclimate to temperature in *A. cristatellus* (Rogowitz 1996b). Taken together these findings suggest that individuals of *A. cristatellus* from the island of Puerto Rico are unable to exhibit a rapid physiological acclimation response to changing climatic conditions.

We are somewhat at a loss to explain the overall increase in water loss rates between Experiment 1 and Experiment 2. It is possible that the physiological ability to resist water loss decreases with time in captivity. Current empirical evidence addressing

this point is lacking in anoles and lizards in general; however, the mass of lizards did not change significantly from Experiment 1 to Experiment 2 (data not shown), so it is unlikely that overall physiological condition decreased over time. Regardless of the overall difference in water loss rates between Experiment 1 and Experiment 2, xeric lizards lost water more slowly than mesic lizards when maintained under identical conditions in both experiments and water loss rates did not differ between individuals from the same habitat type placed under different conditions in Experiment 2. Thus, our data clearly suggest that the populations differ in their ability to resist water loss and that *A. cristatellus* cannot physiologically acclimate to different hydric regimes.

Our results are consistent with previous research on West Indian anoles in suggesting that acclimation is not sufficient to account for patterns of water loss rate variation found between species or populations. In the few species studied thus far, negative correlations between habitat aridity and water loss rates have been found even if lizards from each population are exposed to identical climatic conditions preceding water loss measurement (Hertz *et al.* 1979; Hertz 1980; Perry *et al.* 2000). A series of previous studies has suggested that geographic variation in water loss rates of *A. cristatellus* from the British Virgin Islands is at least partially explained by acclimatory responses to climatic variation (Dmi'el *et al.* 1997; Perry *et al.* 1999, 2000); however, no acclimation was detected when individuals from different regions were placed in a common garden (an enclosure placed in the field)(Perry *et al.* 2000). A field common

garden experiment has been used to address acclimation in only one other tropical *Anolis*, the Lesser Antillean *A. roquet* (Hillman *et al.* 1979). In this study, water loss rates of lizards from xeric, intermediate, and mesic habitats were shown to decrease after being placed in a common garden (a greenhouse in the field). However, in this study the acclimation conditions were unknown because the climatic conditions in the enclosure were not controlled nor measured systematically (Hillman *et al.* 1979). Thus, whether or not the changes observed were in the direction predicted by the acclimatory conditions is unknown. In general, our laboratory data and those of Perry *et al.* (2000) suggest that West Indian *Anolis* are unable to acclimate to changes in hydric conditions. In this respect tropical anoles appear to differ from the temperate *A. carolinensis*, which shows a reduction in cutaneous water loss rates after only 1 week of exposure to low-humidity conditions (Kobayashi *et al.* 1983; Kattan & Lillywhite 1989). Further research needs to evaluate if conditions experienced during embryonic or juvenile stages can contribute to intraspecific differences in physiological traits in anoles (Warner & Andrews 2002).

Our results suggest two non-mutually exclusive mechanisms by which organisms from arid environments can limit rates of water loss. The first is to simply reduce the rate at which water evaporates across the skin (Tables 2, 3). This is generally achieved by increasing the lipid content of the integument (Lillywhite 2006), although variation in the size and morphology of scales might also contribute to differences in water loss (Malhotra & Thorpe 1997; Thorpe *et al.* 2005; Calsbeek *et al.* 2006). However,

in anoles there is no clear pattern with regard to scale size or number and habitat climatic conditions (reviewed in Malhotra & Thorpe 1997; Calsbeek *et al.* 2006). In addition, a study evaluating geographic variation in *A. cristatellus* scalation found no differences in scale size or total scale number between the populations we sampled (Heatwole 1976). The second is to attain a larger size. Desiccation tolerance is often measured as the vital limit, or the percent of original body mass at which an organism dies from dehydration (Hertz *et al.* 1979; Hertz 1980). Larger individuals have a lower surface-area to volume ratio, so all else being equal, a large individual will approach its vital limit more slowly than a small individual will (Hertz 1980). In our sample, *A. cristatellus* from the xeric habitat were larger than those from the mesic habitat when comparing within sexes (t test, $P < 0.001$ for both males and females) (this pattern also holds when utilizing a larger data set, M. Leal and A.R. Gunderson, unpubl. data).

We used data from Experiment 1 to predict how long an “average” male and female individual from each habitat would be able to survive under the experimental conditions used in our study, using a previously published vital limit of 67% of body weight for *A. cristatellus* (Hertz 1979). An average xeric male would survive over 2 days longer than an average mesic male, whereas an average xeric female would survive almost one full day longer than an average mesic female (Table 2). Due to their small size, females are at a significant survival time disadvantage when compared to males. In fact, a mesic male is predicted to survive 2 days longer than a xeric female. This raises

the question of how females, not to mention hatchlings of either sex, deal with this inherent disadvantage of small size. One possibility is that females and smaller individuals seek out areas that are more humid and/or cooler than large males. However, our microclimate measurements showed that neither air temperature nor relative humidity differed between the perch sites used by males and females (all $P > 0.05$, Table 1). In addition, there is no significant difference in body temperatures of male and female *A. cristatellus* (A.R. Gunderson and M. Leal, unpubl. data). Females may also have a lower vital limit or excrete smaller relative amounts of water. How smaller lizards deal with water loss rates in anoles is an open question that deserves further investigation.

Our results demonstrate that adult individuals of *A. cristatellus* are unable to show an acclimatory response to changes in humidity regimes, suggesting that phenotypic plasticity is not the main mechanism leading to differences in water loss rates between individuals inhabiting distinct ecological conditions. Thus, differences in desiccation resistance among populations of *A. cristatellus* may have a genetic basis. Further work is required to understand the interactions between plasticity and heritable variation in physiological traits in the adaptive radiation of anoles, in which interspecific differences in physiology are well accepted as a major contributor to species diversity by allowing species to differentiate along a climatic niche axis (Losos 2009).

2. Geographic variation in vulnerability to climate warming in a tropical Caribbean lizard¹

2.1 Summary

Rising global temperatures are predicted to impact organisms in diverse ways. For ectotherms, recent broad-scale analyses have predicted global patterns of vulnerability to warming, with tropical species at higher risk of detrimental effects than temperate species. However, vulnerability results from complex interactions between environment, physiology and behavior. For species that inhabit a diversity of habitat types, these interactions may change across their range.

We measured operative thermal environments (T_e) and body temperatures (T_b) of the tropical Caribbean lizard *Anolis cristatellus* at nine sites representing two habitat types: mesic and xeric forest. The thermal sensitivity of whole-organism physiological performance (i.e. sprint speed) of one mesic and one xeric population was also measured. Thermal and performance data were integrated to determine how habitat thermal variation, behavioral thermoregulation and thermal physiology influence current physiological performance capacity in the field. We then evaluate if habitat suitability and physiological capacity would change assuming climate warming of 3 °C over the next century.

¹ This chapter has been published in the journal *Functional Ecology*.

The mean T_e of the xeric habitat was 4.5 °C warmer than that of the mesic habitat. However, behavioral thermoregulation by xeric lizards led to lesser differences in T_b (3.5 °C) between habitat types. The thermal sensitivity of sprint performance was similar for mesic and xeric lizards, and lizards from both habitats maintain sprint capacities near 100%. Climate warming is predicted to influence mesic and xeric lizards differently. Xeric lizards currently live in a thermal environment near their upper temperature threshold, while mesic lizards do not. As a result, the number of suitable perch sites is predicted to decrease dramatically in the xeric but not the mesic habitat. In addition, the physiological capacity of mesic lizards is predicted to increase by approximately 4%, whereas a decrease of approximately 30% is predicted for xeric lizards.

We characterized variation in the current biophysical and ecophysiological conditions experienced by *A. cristatellus* by integrating fine-scale measurements of thermal microhabitats with data on body temperatures and physiological performance capacities. These data allowed us to explicitly demonstrate how variation in these parameters can influence population susceptibility to climate warming across a species range and highlight the utility of a mechanistic approach in studies of global climate change.

2.2 Introduction

Environmental temperature variation is extensive across the geographic range of many organisms. Ectotherms, which cannot produce appreciable physiological heat, are particularly sensitive to this thermal variation (Porter & Gates 1969). As such, knowledge of variation in the thermal environment throughout an ectothermic species' range can greatly contribute to our understanding of that species' ecology (Kearney & Porter 2009). For example, studies incorporating thermal variation across a species' range lay the groundwork for understanding variation in thermoregulatory patterns (Huey 1974; Adolph 1990; Freidenburg & Skelly 2004), life-history strategies (Kingsolver 1983a; Dunham *et al.* 1989; Angilletta 2001) and intra- and interspecific variation in thermal physiology (Crowley 1985; Van Damme *et al.* 1990; Willett 2010).

The need to characterize the thermal niche has become increasingly relevant in light of rapid increases in global temperature associated with anthropogenic climate change (IPCC 2007). Several broad-scale analyses of terrestrial ectotherms have suggested global patterns of susceptibility to this warming (e.g., Deutsch *et al.* 2008; Huey *et al.* 2009; Kearney *et al.* 2009; Dillon *et al.* 2010; Sinervo *et al.* 2010; Duarte *et al.* 2012). In general, these studies predict that tropical ectotherms are more susceptible to increasing temperatures than their temperate counterparts, for two reasons (but see Bonebrake & Mastrandrea 2010; Clusella-Trullas *et al.* 2011). First, tropical ectotherms tend to live in thermal environments closer to their upper temperature thresholds.

Second, tropical ectotherms may have less potential to behaviorally buffer themselves from increasing temperatures. For example, among lizards, the thermal safety margin for physiological performance (i.e. the difference between the optimal performance temperature and environmental temperature) decreases with decreasing latitude such that tropical lizards usually have lower capacities to tolerate warming than temperate-zone lizards (Huey *et al.* 2009). Similarly, the reproductive rates of most tropical insects are predicted to decrease because of climate warming, whereas insects in temperate regions are generally predicted to experience increases in reproductive rate (Deutsch *et al.* 2008). However, because of the scale of analysis, these studies often exclude aspects of the thermal ecology of individual species that may increase or decrease their susceptibility to warming, such as fine-scale geographic variation in thermal environment and/or geographic variation in the temperature-dependence of whole-organism performance.

In recent years, characterization of the thermal niche has been carried out mostly using modelling approaches that either correlate climatic data with current distribution data (i.e. correlative models), or integrate physiological data with climatic data to estimate habitat and body temperatures to predict where organisms can occur (i.e. mechanistic models; reviewed in Kearney & Porter 2009; Buckley *et al.* 2010). These approaches have a number of benefits, as they allow estimation of climatic niches for species for which ecological and physiological data are unavailable (primarily

correlative models) or can be used to predict thermal conditions and their physiological consequences for widely distributed species for which direct measurements across the range are impractical (mechanistic models). However, a limitation of those approaches is that body temperatures and operative thermal environments of the organisms in question are not directly measured. Instead, estimated temperatures are often derived from environmental measurements taken at macro-scales that do not necessarily translate to the conditions experienced by organisms in their microhabitats (Chown & Terblanche 2006; Huey *et al.* 2009; Helmuth *et al.* 2010; Sears *et al.* 2011). Furthermore, although behavioral thermoregulation is pervasive among ectotherms (Angilletta 2009), these methods do not measure the extent to which organisms behaviorally thermoregulate across their range, and often ignore behavioral thermoregulation or assume the degree to which it occurs.

The thermal niche can also be characterized by integrating body temperature measurements of free-ranging animals with fine-scale estimates of their operative thermal environment (Christian & Weavers 1996). Contrary to broad-scale modelling, these approaches focus on comprehensive measurement of thermal environments at the scale at which organisms experience them (Gates 1980; Helmuth *et al.* 2010), and many were explicitly developed to detect and estimate the extent of behavioral thermoregulation. Thus, these approaches provide a valuable tool with which to explore the complex interactions between thermal environment, thermal physiology and

behavior that may influence the ability of species to persist in the face of climate warming (Huey *et al.* 2003; Kearney *et al.* 2009). Despite the advantages, studies of this kind are rarely used to evaluate the possible impacts of climate change.

Here, we implement the ‘null model’ approach following Hertz *et al.* (1993) and measure geographic variation in body temperature, operative thermal environment and behavioral thermoregulation in the tropical lizard *Anolis cristatellus*. *Anolis cristatellus* is an arboreal lizard that occupies two distinct habitat types on the Caribbean island of Puerto Rico: mesic (moist, closed-canopy forest) and xeric (dry, relatively open-canopy forest). Tropical lizards are predicted to be more vulnerable to climate change than their temperate-zone counterparts (Huey *et al.* 2009; Kearney *et al.* 2009); however, within the tropics, closed-forest species are predicted to be more susceptible than open-forest species (Huey *et al.* 2009). Here we test this prediction in a species that occupies both habitat types. In doing so, we evaluate how the occupation of different habitats may influence the impacts of climate warming on tropical species.

To achieve this goal, we sampled lizard body temperatures (T_b) and estimated operative thermal environments using copper lizard models (T_e) at nine sites across Puerto Rico (four mesic and five xeric), covering a large proportion of this species’ range (Figure 1). Measurements of T_b and T_e were used to evaluate patterns of behavioral thermoregulation among these sites (Hertz *et al.* 1993). To estimate the impact of temperature on physiological capabilities, we estimated thermal performance curves for

sprint speed for *A. cristatellus* from both mesic and xeric habitats. Locomotor performance (i.e. sprint speed) is an ecologically relevant temperature-dependent performance trait (Bennett 1980; Irschick *et al.* 2008) that is commonly used as a physiological parameter to explore the impact of climate warming on ectotherms, including lizards (Buckley 2008; Kearney *et al.* 2008; Huey *et al.* 2009), and has been used as an estimate of general physiological sensitivity to temperature (Huey *et al.* 2009). We then combined data for T_e , T_b , and physiological sensitivity to temperature to estimate geographic variation in susceptibility to climate warming in *A. cristatellus* across Puerto Rico, using three metrics: (i) warming tolerance, (ii) predicted changes in the thermal suitability of each habitat type assuming a 3°C increase in T_a and (iii) predicted changes in mean whole-organism physiological performance capacity of lizards assuming a 3°C increase in T_a .

2.3 Methods

2.3.1 Study species and study sites

Anolis cristatellus is a small-to-medium sized grey-brown, sexually dimorphic, arboreal lizard typically found on the ground or on perches up to 2 m in height (Rand 1964). *Anolis cristatellus* is endemic to the islands of the Greater Puerto Rican Bank and was historically found from sea level to mid-elevation (<800 m, Rand 1964) throughout the island of Puerto Rico, including both mesic and xeric habitats (Heatwole 1976; Hertz 1992a).

We collected thermal data at nine relatively undisturbed sites across Puerto Rico, four mesic and five xeric (see Figure 1 for locations, see Table 4 for sample sizes). The mesic habitat was sampled at Bosque Estatal de Guajataca (M1: 18°25.236 N; 66°58.010 W; elevation 200 m), Bosque Estatal de Cambalache (M3: 18°26.998 N; 66°35.642 W; elevation 10 m), Bosque Estatal La Vega (M4: 18°24.862 N; 66°20.252 W; elevation 35 m) and a privately owned forest reserve adjacent to the Mata de Plátano field station (M2: 18°24.847 N; 66°43.697 W; elevation 135 m). The xeric habitat was sampled at two sites within the Bosque Seco de Guánica (X2 17°56.711 N; 66°56.308 W, X3: 17°58.246 N; 66°52.236 W, elevation 5 m for both sites), Bosque Estatal de Aguirre (X4: 17°56.348 N; 66°12.100 W, elevation 2 m), Bosque Estatal de Boquerón, Morillos de Cabo Rojo area (X1: 17°57.239 N; 67°11.920 W, elevation 2 m) and a U.S. Naval base, Roosevelt Roads (X5: 18°13.660 N; 65°35.667 W, elevation 10 m). All of the mesic sites lie within Holdridge's Subtropical Moist Forest life-zone (Ewel & Whitmore 1973; Daly *et al.* 2003) and are part of a contiguous area of predominantly mesic forest that extends across most of the northern and western coastal regions of the island. Four of the xeric locations (X1–X4) are part of a continuous swath of xeric habitat that runs along much of the southern coast of Puerto Rico. The other xeric site (X5) is separated from the others by mesic habitat and is part of 'Las Cabezas de San Juan', a region of xeric habitat in the north-eastern corner of the island. All of the xeric sites lie within Holdridge's Subtropical Dry Forest life-zone (Ewel & Whitmore 1973). In general, the xeric habitat receives less

rainfall than the mesic habitat, by as much as 10 cm per month (Daly *et al.* 2003; Brandeis *et al.* 2009).

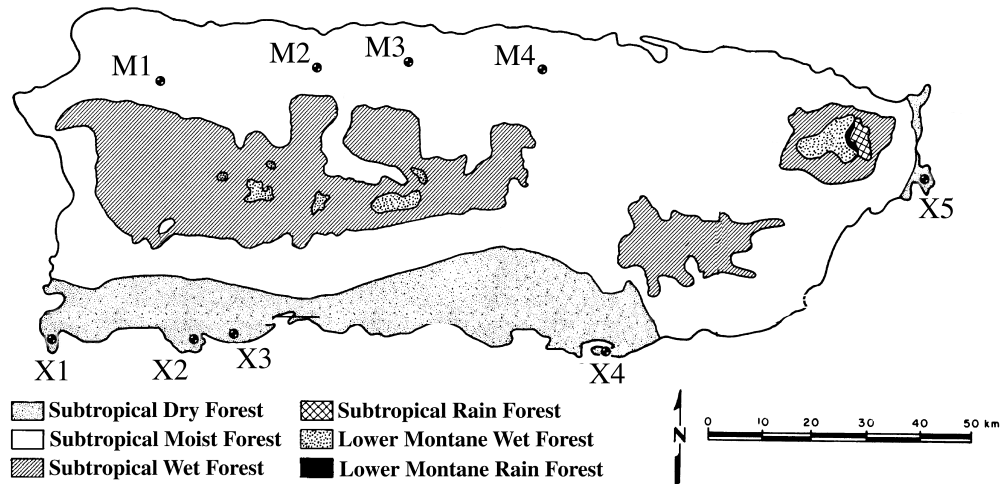


Figure 1. Map showing the location of the nine sites (populations of *A. cristatellus*) sampled in this study and life zones present on the island of Puerto Rico (adapted from Ewell & Whitmore 1973). M1 = Guajataca, M2= Mata de Plátano, M3= Cambalache, M4= La Vega, X1= Cabo Rojo, X2= Guanica 1, X3= Guanica 2, X4= Aguirre, X5= Roosevelt Roads.

2.3.2 Lizard and copper model thermal data

We measured T_b and T_e (copper model temperatures) at each of the nine sites between July 7 and August 22, 2009. Thermal data were also collected at sites M3 and X4 from 14 to 21 December 2009. Each location was sampled for 3 days from 07:00 to 18:00

h, totaling 33 h of sampling effort per site (i.e. 3 h of sampling per hour of the day), per season. However, site M3 was sampled for 30 h over 3 days in July, and site X5 was sampled for 19 h over 2 days. Our sampling effort should produce a representative picture of the thermal environments experienced by lizards at each site in a given season because of the low thermal variability of tropical climates (Janzen 1967). This is evident in our data. Among sites within a given habitat type sampled in the same season (July and August), all of which were sampled on different days, pair-wise differences in mean operative temperatures were never more than 1 °C (Table 4). Previous studies in Puerto Rico have found that even within sites, maximum seasonal differences in mean air temperature are about 3 °C (Brandeis *et al.* 2009; a similar pattern was found for T_b and T_e in this study, see seasonal data in the Results). Data were not collected while it was raining because lizards are inactive. In total, the mesic and xeric habitats received 129 and 151 h of sampling effort, respectively, throughout July and August. We focused our sampling efforts during the months of July and August because this period coincides with high rates of reproductive activity of *A. cristatellus* across Puerto Rico (Gorman & Licht 1974).

At each site, a location in the forest was haphazardly chosen as a starting point for sampling for a given day. From this point, a compass direction was taken from a random number table. This compass direction was used as a transect line along which sampling occurred. The transect line was walked until an impassable barrier was

reached (e.g. ocean, fence, road, cliff), at which point another random compass direction was then taken as a new transect line (under the restrictions that it moved away from the impassable barrier and, to avoid re-sampling the same individuals that had previously been disturbed, was not 180° from the previous transect line). Thus, we sampled large areas by crisscrossing the forests along multiple, randomly chosen transects.

At each site, we walked slowly along transects looking for lizards. When a lizard was spotted, we attempted to capture it by noosing to measure its T_b . We did not sample lizards that moved from either sun to shade or shade to sun during the capture process. Immediately upon capture, T_b was measured cloacally with a thermocouple probe connected to a digital thermocouple thermometer (Omega Engineering Inc., Stamford, CT, USA) accurate to 0.1 °C. The snout-to-vent length (SVL) of each lizard was also measured, and all individuals were released at the site of capture. Both males and females were sampled and sexes did not differ in T_b in either habitat (Wilcoxon rank sum tests, both $P > 0.05$). Thus, data for males and females were pooled for all analyses. Mesic individuals had a mean (\pm SD) SVL of 47.9 ± 7.9 mm, while xeric individuals had a mean SVL of 53.3 ± 9.2 (see Table 4). After each lizard was sampled, we took a T_e measurement by recording the temperature of a copper lizard model at a random location within 8 m of the capture site. We used painted, hollow copper models with a thermocouple affixed inside and shaped with a mold of *A. cristatellus*. The models are

designed to have a low specific heat capacity, and thus their temperature rapidly reaches equilibrium under field conditions (see Hertz 1992b). In practice, we concluded that a model reached equilibrium when its temperature did not change (at an accuracy of 0.1 °C) for 10 s. Our models were constructed by P.E. Hertz and have been used in previous studies of *A. cristatellus* thermal ecology (see Hertz 1992a; Hertz 1992b for a detailed description of the models). To determine the copper model location, we generated a random number table that gave random compass directions (1–360° at one degree intervals), distances (0–8 m at 0.5 m intervals) and perch heights (0–200 cm at 1 cm intervals). Two meters was chosen as the maximum height because *A. cristatellus* is rarely found above this height (Rand 1964). We chose eight meters as the maximum distance because we have rarely observed an individual moving more than 8 m in a single bout in the field (A. R. Gunderson, pers. obs.). Using this sampling scheme, a model temperature was taken approximately 3–4 min after each lizard temperature was collected. These model temperatures provide an estimate of the T_e distribution at each site. For each lizard and model, we also recorded the immediate weather conditions (cloudy, sun visible behind clouds, sunny) and basking status (not basking: $\leq 25\%$ of body in direct sunlight; basking: $> 25\%$ of body in direct sunlight).

Neither T_b nor T_e data were normally distributed; therefore, we used nonparametric models (Kruskal–Wallis and Wilcoxon sum rank tests) to test for differences between populations and habitats.

2.3.3 Estimating the extent of behavioral thermoregulation

For each site, we calculated the extent of behavioral thermoregulation using two different metrics: E , calculated as $E = 1 - d_b/d_e$ (Hertz *et al.* 1993), and $d_e - d_b$, or the absolute temperature difference of mean d between lizards and models (Blouin-Demers & Weatherhead 2001). The variables d_b and d_e describe the mean deviation of T_b and T_e from the preferred temperature (i.e. set point) range, respectively. Preferred temperatures, calculated as the central 50% of body temperatures chosen by lizards in a laboratory thermal gradient, were previously reported for one mesic site (approximately 10 miles north-east of our site M4, $N = 6$) and one xeric site (our site X3, $N = 6$; Huey & Webster 1976; Hertz *et al.* 1993). The preferred temperature ranges differed slightly between populations of *A. cristatellus* (mesic preferred temperature range = 28.6–30.7°C; xeric preferred temperature range = 28.6– 30.9°C). Therefore, we used the mesic preferred temperature range for d calculations at all mesic sites, and the xeric preferred temperature range for d calculations at all xeric sites.

Two complementary analyses were used to test whether lizards at a particular site are non-randomly utilizing available thermal resources and, thus, behaviorally thermoregulating (Hertz *et al.* 1993). First, we used Wilcoxon's sum rank tests to compare T_b to T_e within each site. Second, we conducted G-tests to determine whether basking rates differed between lizards and copper models (i.e. random perch sites).

In addition, to determine whether lizard use of available thermal resources increased their predicted whole-organism performance capacity over null expectations, we used Wilcoxon's sum rank tests to compare the predicted relative performance capacity of lizards and of copper models within a site.

2.3.4 Temperature-dependent whole-organism performance

Male lizards from mesic site M3 and xeric site X3 were collected from 5 to 8 August 2010 and transported to Duke University. In the laboratory, lizards were housed individually in plastic cages (29 x 21 x 21 cm) with a wooden dowel provided as a perch in a climate-controlled growth chamber (28°C, 60% relative humidity) under a 12L:12D light cycle. All lizards were watered daily and fed crickets dusted with calcium powder three times per week.

We calculated sprint speed by conducting frame-by-frame video analysis of lizards running up a wooden racetrack (Husak 2006). The racetrack was 1.5 m in total length, with visible landmarks every 25 cm and was set at a 37° angle. Runs at 19.5 and 32°C were filmed with a Canon ZR 950 camcorder (Canon USA, Inc., Lake Success, NY, USA) at 29.5 frames-per-second. Runs at all other temperatures were filmed at 120 frames-per-second using the Slow Smooth Record function on a Sony HDR-SR11 Handycam (Sony Corporation of America, New York, NY, USA).

Lizards were induced to run, if necessary, by gently tapping their tails. At each test temperature, we analyzed two 'good' runs per lizard, defined as a run in which the

lizard neither stopped nor jumped off of the track. Lizards were run twice per day (once in the morning and once in the afternoon). For 'good' runs, we measured sprint speed over each 25-cm interval and used the fastest interval as an individual's speed for that temperature (following Huey 1983). Each lizard was run at six temperatures: 19.5, 23, 26, 29, 32 and 35°C in the following randomized order: 19.5, 32, 29, 23, 26 and 35°C. The desired body temperature was attained by placing the lizards in either a climate-controlled room or incubator set to the test temperature 1 h prior to a run. We confirmed that ambient temperatures and body temperatures were equivalent at 19.5 and 35°C and are confident that test temperatures and body temperatures are in accordance across the range temperatures used. This is expected for an ectotherm with low rates of water loss in a room with no solar radiation and little to no air-flow (Gates 1980). All runs were conducted 24 h after feeding. To calculate the thermal performance curve, we only included data for individuals that were run at a minimum of five of the test temperatures (lizards were removed from trials if a portion of their tail was lost during the experiment), giving us a sample size of eight mesic and five xeric lizards.

Sprint speed data were used to generate a temperature-dependent sprint performance curve (Figure 2B). At each temperature, mesic and xeric lizards had nearly identical mean relative sprint performance (Figure 2B). Thus, we combined data from mesic and xeric lizards to generate a single thermal performance curve that was used in all whole-organism performance analyses. Following Huey *et al.* (2009), we modelled the

temperature-dependence of sprint performance by combining two different functions.

For the left side of the curve, from the lowest temperature to the optimum temperature,

we fitted the following function to the data: $P(T) = e^{-\frac{(T-T_{opt})^2}{2\sigma^2}}$ (Huey *et al.* 2009), where P

= relative sprint performance, T = temperature, T_{opt} = temperature of optimal

performance, and σ is a term related to the breadth of the curve. We solved for the T_{opt}

and σ terms using a nonlinear least-squares analysis implemented in the R statistical

programming language. For temperatures above T_{opt} , the curve was described by the

function: $P(T) = 1.0 - \left(\frac{T - T_{opt}}{CT_{max} - T_{opt}} \right)^2$ (Huey *et al.* 2009), where CT_{max} is the ‘critical thermal

maximum’, or the upper body temperature at which a lizard loses the ability to right

itself, which is often used as a proxy for the upper lethal temperature threshold (Huey &

Stevenson 1979). We obtained the CT_{max} of *A. cristatellus* from previously published data

(Mesic lizards: N = 10, $\bar{X} = 38.1 \pm 0.4^\circ\text{C}$; xeric lizards: N=11, $\bar{X} = 38.9 \pm 0.3^\circ\text{C}$; Huey & Webster

1976).

The estimated temperature-dependent performance curve was applied to the sampled values of T_b and T_e to estimate the physiological performance consequences of thermal variation across the range of *A. cristatellus*.

2.3.5 Susceptibility to climate warming

We evaluated the susceptibility of *A. cristatellus* to climate warming using three metrics. First, we calculated the ‘warming tolerance’ [i.e. the difference between the

current mean T_e and the CT_{max} (Deutsch *et al.* 2008)] of lizards in both habitat types. We used mean CT_{max} values for *A. cristatellus* from each habitat type reported in Huey and Webster (1976). Next, we estimated whether or not the suitability of the thermal environment in each habitat will change by calculating the percentage of the available thermal environment (i.e. the T_e distribution) that will be within 2 °C of CT_{max} after climate warming. We assume a 3 °C increase in T_a (Buckley 2008; Huey *et al.* 2009; Kearney & Porter 2009; Buckley *et al.* 2010), which is within the range of predicted increases in air temperature in Puerto Rico over the next century (Christensen *et al.* 2007). Furthermore, we assumed that T_e changes at the same rate as T_a . Finally, using the thermal performance curve for sprint speed (Figure 2B), we predicted the change in mean performance capacity of lizards in each habitat from current values, again assuming a 3 °C increase in T_a . We assumed that T_b increases with T_a in a one-to-one relationship, which has been previously demonstrated for two populations of *A. cristatellus* on Puerto Rico (Huey *et al.* 2009). We do not incorporate potential changes in wind-speed into our predictions for T_e and T_b because at present there is no reliable method to predict wind-speed changes over the next century (IPCC 2007).

2.3.6 Statistical analyses

Adjustment of the Type I error rate for multiple tests was carried out using the false-discovery rate method (Whitlock & Schluter 2009). All statistical tests were two-

tailed at $\alpha = 0.05$ and were performed using the R statistical programming language V 2.9.2 (R Development Core Team 2012).

2.4 Results

2.4.1 Operative thermal variation

Mean T_e in the xeric habitat ranged from 32.9 to 33.9°C and did not differ significantly among sites (Kruskal–Wallis sum rank test, chi-squared = 8.30, d.f. = 4, $P = 0.081$), whereas mean T_e across mesic sites ranged from 28.5 to 29.2°C and differed significantly (Kruskal–Wallis rank sum test, chi-squared = 15.53, d.f. = 3, $P = 0.001$; Table 4). However, differences in mesic mean T_e s were small. T_e s at M1 and M2 were each cooler than T_e s at sites M3 and M4 (multiple pair-wise comparisons with false-discovery rate correction for Type I error rate, all $P < 0.05$; Table 4). Combining data within mesic and xeric sites, T_e differed between mesic and xeric habitats. During July and August, the mean T_e of the xeric habitat was significantly warmer than that of the mesic habitat, by approximately 4.5°C (Wilcoxon sum rank test, $P < 0.001$; Table 4).

In December, mean T_e at xeric site X4 (30.1°C) was significantly warmer than mean T_e at mesic site M3 (26.2°C; Wilcoxon sum rank test, $P < 0.001$; Table 4). In addition, mean T_e at both sites was significantly cooler in December than in July and August (Wilcoxon sum rank tests, both $P < 0.001$; Table 4).

Table 4. Summary statistics for *Anolis cristatellus* field thermal data from four mesic sites and five xeric sites in Puerto Rico. N = sample size, T_b = mean lizard body temperature (°C), P = mean relative sprint performance capacity (% of maximum sprint performance), T_e = mean copper model temperature (°C).

<u>Month & Site</u>	<u>N_{lizards}</u>	<u>T_b</u>	<u>P_{lizards}</u>	<u>N_{models}</u>	<u>T_e</u>	<u>P_{models}</u>
July-Aug						
M1	127	28.6 (0.2)	94.3 (0.4)	127	28.5 (0.1)	94.3 (0.3)
M2	94	28.7 (0.2)	94.6 (0.4)	91	28.7 (0.2)	94.6 (0.3)
M3	96	29.2 (0.1)	95.8 (0.2)	95	29.2 (0.1)	95.9 (0.2)
M4	152	29.0 (0.1)	95.2 (0.3)	151	29.0 (0.1)	95.4 (0.3)
Mesic total	469	28.9 (0.1)	95.0 (0.2)	464	28.9 (0.1)	95.0 (0.2)
X1	136	32.6 (0.1)	97.9 (0.3)	137	33.5 (0.2)	87.8 (2.2)
X2	140	32.6 (0.2)	96.9 (0.4)	140	33.1 (0.2)	92.0 (1.2)
X3	116	32.4 (0.2)	97.8 (0.3)	114	33.2 (0.3)	88.1 (2.2)
X4	140	32.8 (0.2)	96.9 (0.4)	138	33.9 (0.2)	85.2 (2.2)
X5	106	32.5 (0.2)	97.3 (0.4)	103	32.9 (0.2)	93.5 (1.6)
Xeric total	638	32.6 (0.1)	97.3 (0.2)	632	33.4 (0.1)	84.7 (1.9)
December						
M3	118	26.3 (0.2)	88.4 (0.6)	117	26.2 (0.2)	88.2 (0.6)
X4	160	29.9 (0.2)	96.2 (0.8)	160	30.1 (0.2)	94.2 (0.9)

2.4.2 Body temperatures

Across xeric sites, mean T_b ranged from 32.4 to 32.8°C (Table 4) and did not vary significantly (Kruskal–Wallis sum rank test, chi-squared = 6.07, d.f. = 4, $P = 0.194$). Across mesic sites, mean T_b ranged only from 28.6 to 29.2°C (Table 4) but nonetheless varied significantly (Kruskal–Wallis rank sum test, chi-squared = 12.71, d.f. = 3, $P = 0.006$). Sites M1 and M2 were each significantly cooler than sites M3 and M4 (all $P < 0.05$), a pattern that is congruent with the differences in operative temperatures between mesic sites.

The mean T_b (32.6°C) of lizards across xeric sites in July and August was significantly cooler than the mean T_e (33.4°C; Wilcoxon sum rank test, $P < 0.001$; Table 4). This pattern was most pronounced at midday (Figure 3). Thus, xeric lizards appear to actively seek cool perch sites during the warmest portion of the day. By contrast, T_b across mesic sites did not differ from T_e ; indeed, combining data for mesic sites, lizards and copper models had identical mean temperatures (28.9°C; Table 4). Accordingly, T_b tracked T_e throughout the day at all mesic sites (Figure 3).

In accordance with the July and August data, habitat differences in lizard body temperature were also present in December. Mean T_b at xeric site X4 was 3.6°C warmer than mean T_b at mesic site M3 (Wilcoxon sum rank test, $P < 0.001$; Table 4, Figure 4). However, unlike the July and August data, T_b did not differ significantly from T_e at either site (Wilcoxon sum rank tests, both $P > 0.05$). In addition, mean T_b at both sites was significantly cooler in December than in July and August, by approximately 3°C (Wilcoxon sum rank tests, both $P < 0.001$; Table 4)

2.4.3 Whole-organism performance capacity

In terms of absolute sprint speed, on average mesic lizards ran faster than xeric lizards at all test temperatures (Figure 2A). However, mesic and xeric lizards ran at nearly identical relative sprint speeds at all temperatures (Figure 2B).

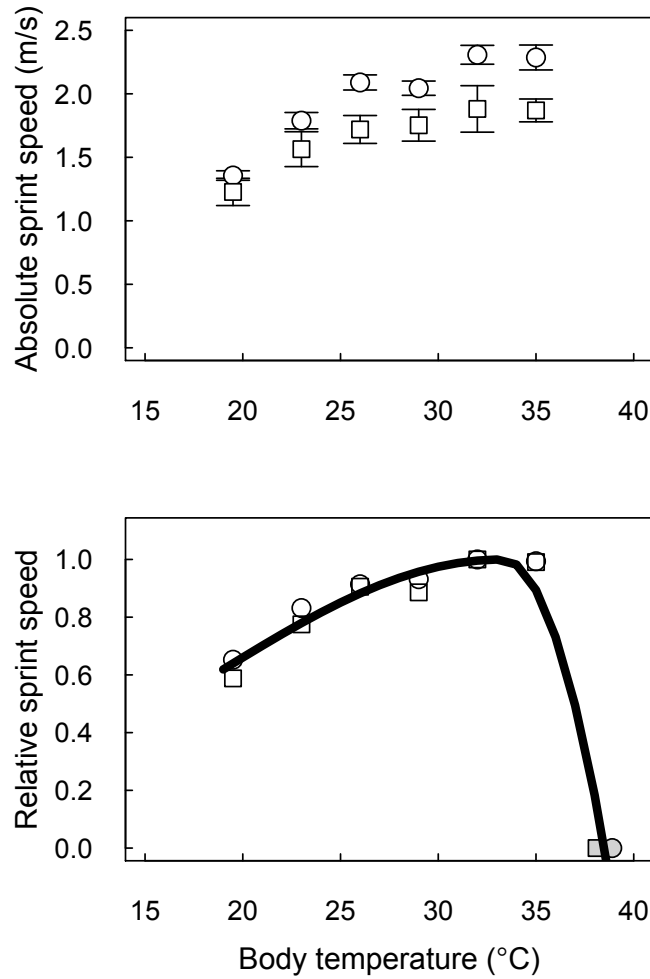


Figure 2. Temperature-dependent sprint performance for a mesic and a xeric population of *Anolis cristatellus*. (A) Mean absolute sprint speed (\pm SE). (B) Relative sprint speed. circles = mesic lizards; squares = xeric lizards. Note: CT_{max} values (shaded symbols) were obtained from Huey & Webster (1976).

Across xeric sites, mean sprint performance capacity of lizards ranged from 96.9% to 97.9%. Within these populations, mean lizard performance capacity ranged from 4.9% to 11.7% higher than mean copper model performance capacity (Table 4,

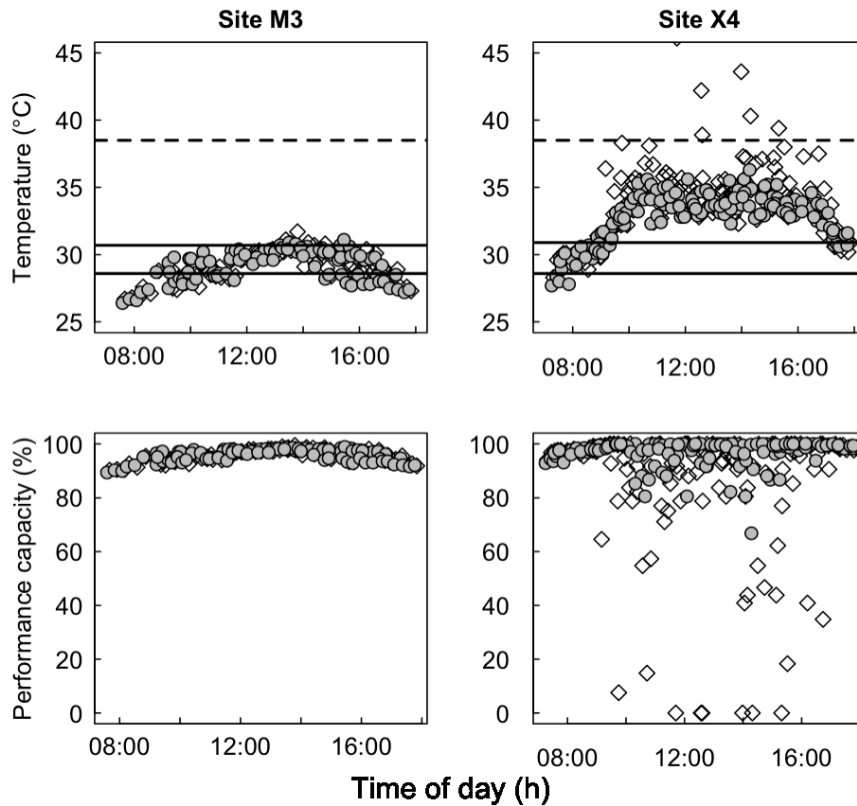


Figure 3. Temperatures and estimated performance capacities for *Anolis cristatellus* (filled circles) and copper models (open diamonds) at one mesic and one xeric site in July and August. Dashed lines indicate the critical thermal maximum (CT_{max}; Huey & Webster 1976). Solid lines encompass the preferred temperature range (Hertz *et al.* 1993).

Figure 3). A closer examination reveals that the difference between lizard and copper model performance capacity was particularly marked at midday (11:00–13:00 h), when lizard mean performance capacity was 8.2–21.4% higher than that of copper models. Across mesic sites, mean sprint performance capacity ranged only from 94.3% to 95.8% (Table 4). Contrary to xeric sites, performance capacities of lizards and models were very similar under mesic conditions (Table 4).

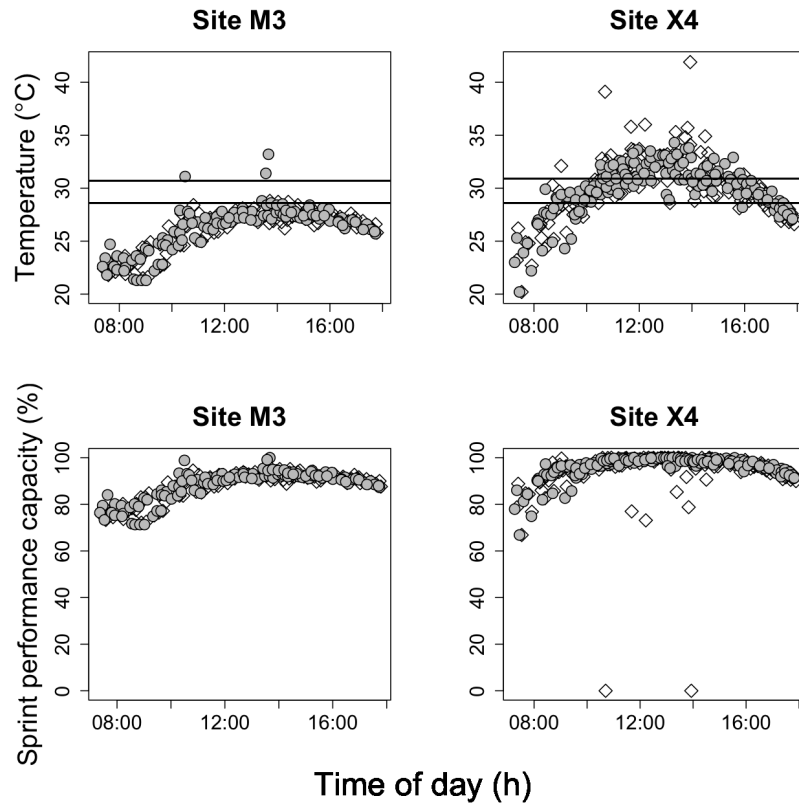


Figure 4. Lizard (filled circles) and copper model (open circles) temperatures and estimated performance capacities at two sites in December. Symbols as in Figure 3.

Lizards and copper models did not differ in performance capacity at either site during December (Wilcoxon sum rank tests, both $P > 0.05$, Table 1). In addition, lizard and copper model performance capacities were significantly lower in December than in July and August for both sites (Wilcoxon sum rank tests, both $P < 0.001$; Table 4).

2.4.4 Behavioral thermoregulation

Within mesic sites, the extent of thermoregulation (E) ranged from -0.19 to -0.03, and $d_e - d_b$ ranged from -0.09 to -0.01 (Table 5). Within xeric sites, E ranged from 0.18 to 0.32, while $d_e - d_b$ ranged from 0.41 to 0.85 (Table 5). Combined with the data on differences between T_b and T_e , these results indicate that xeric lizards behaviorally thermoregulate, while mesic lizards do not. In addition, at four of five xeric sites, lizards basked significantly less often than models (sites X2 – X5, G-tests, all $P < 0.05$), whereas basking rates of lizards and models did not differ significantly at any of the mesic sites (G-tests, all $P > 0.05$).

2.4.5 Susceptibility to climate warming

Across the mesic sites, warming tolerance ranged from 8.9 to 9.6°C ($\bar{X} = 9.3^\circ\text{C}$). Warming tolerance across xeric sites was lower and ranged from 5.0 to 6.0 °C ($\bar{X} = 5.6^\circ\text{C}$).

Combing data within habitat types, a 3°C increase in T_a is predicted to impact the thermal suitability of each habitat type differently. In the xeric habitat thermal suitability is predicted to decrease, with the number of perch sites within 2°C of CT_{\max} increasing from 7% currently to 41%. In contrast, the thermal suitability of the mesic habitat is not predicted to change. We recorded no T_e values within 2°C of CT_{\max} in the current mesic habitat, and no T_e values are predicted to occur within 2°C of CT_{\max} with a 3°C increase in T_a .

Table 5. Summary statistics evaluating the use of behavioral thermoregulation by *Anolis cristatellus* at nine sites across Puerto Rico. Bask = frequency of observations in which over 25% of the body of the lizard (or copper model) was in direct sunlight, d_b = mean absolute deviation of lizard body temperatures from the preferred temperature range, d_e = mean absolute deviation of operative temperatures from the preferred temperature range, E = the effectiveness of behavioral thermoregulation ($1(d_b / d_e)$); (Hertz *et al.* 1993).

<u>Month & Site</u>	<u>Bask_{lizards}(%)</u>	<u>Bask_{models}(%)</u>	<u>d_b</u>	<u>d_e</u>	<u>$d_e - d_b$</u>	<u>E</u>
July-Aug						
M1	16	14	0.72	0.70	-0.02	-0.03
M2	14	8	0.69	0.60	-0.09	-0.14
M3	20	24	0.25	0.25	-0.01	-0.10
M4	13	10	0.55	0.47	-0.09	-0.19
Mesic total	16	14	0.57	0.51	-0.06	-0.11
X1	31	48	1.86	2.71	0.85	0.31
X2	26	46	2.12	2.69	0.57	0.21
X3	14	32	1.77	2.59	0.82	0.32
X4	26	49	2.17	3.13	0.62	0.30
X5	15	36	1.86	2.27	0.41	0.18
Xeric total	22	42	1.97	2.70	0.73	0.27
December						
M3	8	19	2.45	2.43	-0.03	-0.01
X4	37	42	1.00	1.31	0.30	0.23

Finally, assuming a 3°C increase in T_a , lizards in the xeric habitat are predicted to experience a decrease in mean performance capacity ranging from 26% to 32%, depending on the site (Figure 5). In contrast, lizards in the mesic habitat are predicted to experience an increase in mean performance capacity (~3.5%) with the same increase in T_a (Figure 5).

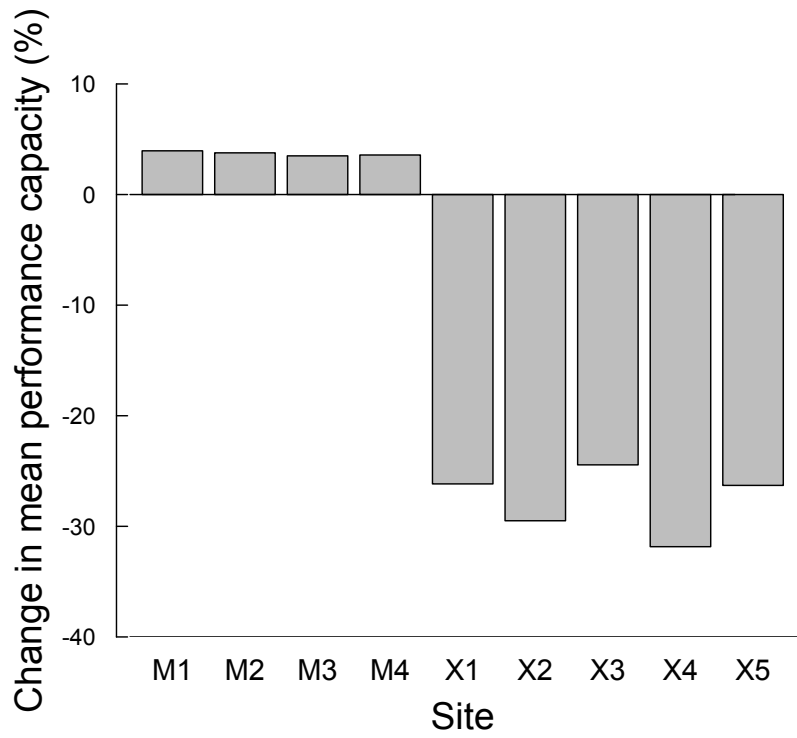


Figure 5. Estimated impact of climate warming (i.e., a 3 °C increase in T_a) on mean performance capacity of *Anolis cristatellus* at nine sites across Puerto Rico. Mesic sites are designated with an 'M', xeric sites are designated with an 'X'.

2.5 Discussion

We found that xeric and mesic habitats present two distinct thermal niches for *A. cristatellus* and that differences in thermoregulatory strategies between habitats are associated with differences in operative temperatures (T_e). Nevertheless, we found no difference in the thermal sensitivity of whole-organism performance (i.e. sprint speed) of *A. cristatellus* occupying these habitats (Figure 2B). Furthermore, individuals from both

habitats achieve high performance capacities under current conditions (Table 4); however, global warming is predicted to impact the performance capacities of mesic and xeric lizards differently.

The mean T_e of the xeric habitat (33.4°C) was 4.5°C warmer than that of the mesic habitat (28.9°C), and T_{es} in the xeric habitat sometimes reached lethal temperatures, which we never observed in the mesic habitat. The difference between xeric and mesic habitats in T_e was also present across seasons (Table 4). Taken together these findings demonstrate that populations of *A. cristatellus* experience two distinct thermal niches, in which xeric habitats are significantly warmer than mesic habitats. Previous studies conducted throughout the Caribbean have also found that xeric and mesic habitats provide distinct thermal niches for lizards, including anoles and *Sphaerodactylus* (e.g., Ruibal 1961; Huey & Webster 1976; Álvarez 1992; Hertz 1992a; Hertz 1992b; Knouft *et al.* 2006). In addition, mesic and xeric habitats on Puerto Rico have been shown to provide distinct physical environments with respect hydric conditions (see Chapter 1, Gunderson *et al.* 2011) and spectral characteristics (Leal & Fleishman 2004).

The pattern observed in T_e was also present in T_b , but to a lesser degree because of behavioral thermoregulation by xeric lizards. On average, xeric lizards were 3.5°C warmer than mesic lizards (Table 4). Additionally, mesic lizards were able to remain within their preferred temperature range for the majority of the day, whereas xeric lizards were unable to attain preferred temperatures for most of the day (Figure 3).

Although lizards from xeric and mesic sites had lower T_b s in December, xeric lizards remained significantly warmer than mesic lizards (Table 4). Therefore, we found that differences in T_b are persistent across season, which is congruent with the results reported by Hertz (1992b).

Although mesic and xeric habitats present two distinct thermal regimes, lizards from both mesic and xeric habitats are able to achieve high physiological performance capacities (means over 90%). How is it that lizards with the same thermal sensitivity can share similarly high performance capacities despite living under distinct operative thermal environments? Two factors can contribute to this finding: behavioral thermoregulation and the shape of the thermal sensitivity curve. Mesic lizards are thermoconformers, but xeric lizards behaviorally thermoregulate by seeking out the coolest available perch sites, thereby reducing the difference in the thermal conditions experienced by lizards in each habitat. If lizards in both habitats were thermoconformers, mean T_b s would be expected to differ by 4.5°C, but instead differ by 3.5°C. This pattern, known as the 'Bogert Effect', is commonly seen among populations occupying different climatic regions (Bogert 1949; Huey *et al.* 2003).

In addition, *A. cristatellus*, like most organisms (reviewed in Angilletta 2009), has a broad plateau of low thermal sensitivity at body temperatures below the optimal temperature for performance ($T_{opt} = 33.3$; Figure 2B). Both mesic and xeric lizards experience mean T_b s below T_{opt} , (Table 4) and thus the relatively large difference in T_b

translates into a minor difference in physiological performance. The complex interactions between operative thermal environment, behavior and thermal physiology underline the advantages of integrating all three of these factors when evaluating the thermal niche.

At the microhabitat level, the xeric habitat had greater variation in T_e than the mesic habitat (xeric T_e range = 21.5°C; mesic T_e range = 8.5°C; Figure 3). A potential consequence of reduced thermal variation at mesic sites is an increase in the cost of behavioral thermoregulation, as perch sites that deviate substantially from the mean T_e are rare and require high energetic expenditures to occupy them (Huey 1974; Huey & Slatkin 1976; Huey & Webster 1976; but see Angilletta 2009 for a model of how the spatial distribution of available operative temperatures can influence thermoregulatory costs). The constraint placed on behavioral thermoregulation by low thermal variability within tropical forests has been suggested to increase the susceptibility of tropical lizards to climate warming (Huey *et al.* 2009). However, for populations of *A. cristatellus* inhabiting mesic forest, low thermal variability does not appear to pose a problem, as we predict that mesic lizards will experience a slight increase in physiological performance capacity with warming.

In contrast, the xeric habitat is more thermally variable, and thus, the cost of attaining body temperatures that deviate from the mean T_e is likely reduced. Xeric lizards do thermoregulate and, in doing so, increase their physiological performance

capacity relative to a hypothetical thermoconformer (i.e. copper models) at the same site (Figure 3, Table 4). However, we predict that *A. cristatellus* in the xeric habitat are more susceptible to climate warming than those in the mesic habitat. In the case of xeric populations, the thermal environment is already warm relative to their thermal sensitivity (Figure 3, Table 4), a pattern predicted to be common for tropical lizards (Kearney *et al.* 2009). Most likely in response to this relatively warm environment, *A. cristatellus* in the xeric habitat already utilize the coolest perches available during the warmest times of the day. Thus, behavioral thermoregulation may have limited potential to buffer xeric lizards from increasing T_b s as environmental temperatures rise over the next century.

At present, *A. cristatellus* living in the xeric habitat have a warming tolerance that is 3.7°C lower than the warming tolerance of lizards from the mesic habitat, and a 3°C increase in T_a is predicted to decrease the thermal suitability of the xeric environment. In addition, xeric lizards are predicted to experience a decrease in physiological performance capacity of approximately 30% with a 3°C increase in T_a . This drop in performance of xeric lizards would result from their body temperatures exceeding the optimal performance temperature, placing them on the steep slope on the right side of the asymmetrical sprint performance curve (Figure 2B). As a consequence of changes in T_e and performance capacity, xeric lizards will likely be forced to be inactive in cool retreats for several hours a day, reducing rates of energy acquisition and possibly

leading to population collapse (Dunham 1993; Huey *et al.* 2009; Sinervo *et al.* 2010). Furthermore, at some xeric sites, *A. cristatellus* is found sympatrically with *A. cooki*, a species that prefers warmer microhabitats and is more heat tolerant (Huey & Webster 1976; Hertz 1992a). These species use the same structural niche and compete for resources (Ortiz & Jenssen 1982), and thus increased inactivity of *A. cristatellus* might provide a competitive advantage to *A. cooki*.

It has been suggested that the negative effects of climate warming may be mitigated by shifts in phenology (Parmesan 2006; Bradshaw & Holzapfel 2008; Willis *et al.* 2008; Pau *et al.* 2011). For example, under climate warming periods that were previously unfavourable for activity (i.e. too cold) may become favourable, therefore buffering populations from decreased activity during times that become too warm. However, this mechanism of coping with warming is unlikely to buffer populations of *A. cristatellus* in xeric habitats, for two reasons. First, *A. cristatellus* is a diurnal lizard, and individuals are already active from sun-up to sun-down. Therefore, increasing temperatures are unlikely to affect daily start and end times for activity. Second, like many tropical organisms, *A. cristatellus* is reproductive year round (Gorman & Licht 1974). Thus, temperature change cannot affect start and end times of reproduction. In mesic habitats reproductive effort (measured as the % of females gravid in point estimates) remains high (>65%) during the nine warmest months of the year and on average does not decrease below 30% during the coldest month (Gorman & Licht 1974).

Although data for xeric *A. cristatellus* are limited, they appear to experience even less seasonal variation in reproductive effort (Gorman & Licht 1974).

The prognosis for mesic *A. cristatellus* is more positive. With a 3°C increase in T_a , mesic lizards will still have a warming tolerance higher than the current value for xeric lizards, and the thermal suitability of the mesic habitat is not predicted to decline. In addition, because mesic lizards currently have T_b s that place them in the middle of the broad lower shoulder of the sprint performance curve (Figure 2), a 3°C increase in T_a is predicted to increase mean performance by approximately 3.5% (Figure 5). Furthermore, contrary to *A. cristatellus* in the xeric habitat, *A. cristatellus* in the mesic habitat are not sympatric with congeners that use the same structural niche but prefer a warmer thermal niche. If community composition stays the same, mesic *A. cristatellus* are not likely to face the potential for competitive exclusion under climate warming.

Our results reinforce the benefits of a mechanistic approach, in which physiological traits are considered in concert with operative thermal environments (Helmuth *et al.* 2005; Kearney & Porter 2009). For example, our prediction that mesic and xeric *A. cristatellus* will be differentially affected by climate warming resulted from combining thermal sensitivity, body temperature and operative temperature data. In addition, we further demonstrate the advantage of direct measurements of the thermal environment and body temperatures (see also Gilman *et al.* 2006), as these allow one to elucidate variation in patterns of behavioral thermoregulation, which can be important

for species persistence as thermal conditions change (Huey *et al.* 2003; Kearney *et al.* 2009; Huey *et al.* 2010).

3. Rapid change in the thermal tolerance of a tropical lizard¹

3.1 Summary

The predominant view is that the thermal physiology of tropical ectotherms, including lizards, is not labile over ecological timescales. We used the recent introduction (~35 years ago) of the Puerto Rican lizard *Anolis cristatellus* to Miami, Florida, to test this thermal rigidity hypothesis. We measured lower (critical thermal minimum [CT_{\min}]) and upper (critical thermal maximum [CT_{\max}]) thermal tolerances and found that the introduced population tolerates significantly colder temperatures (by ~3 C) than does the Puerto Rican source population; however, CT_{\max} did not differ. These results mirror the thermal regimes experienced by each population: Miami reaches colder ambient temperatures than Puerto Rico, but maximum ambient temperatures are similar. The differences in CT_{\min} were observed even though lizards from both sites experienced nearly identical conditions for 49 days before CT_{\min} measurement. Our results demonstrate that changes in thermal tolerance occurred relatively rapidly (~35 generations), which strongly suggests that the thermal physiology of tropical lizards is more labile than previously proposed.

¹ This chapter has been published in the journal *The American Naturalist*.

3.2 Introduction

The general view is that climate change will have a major impact on biodiversity by increasing the extinction risk of many species or changing their distributions (Pounds *et al.* 1999; Moritz *et al.* 2008). This view is based on the implicit assumption that species are relatively fixed entities, unable to respond to rapid changes in ecological conditions, including climatic variables, over an ecological timescale. Nevertheless, evidence that some organisms are able to respond to climatic changes over short timescales has begun to emerge (e.g., Grant & Grant 2002; Walther *et al.* 2002; Bradshaw & Holzapfel 2006). However, the rate at which species respond to climate change may be clade specific (Willis *et al.* 2008; Huey *et al.* 2009).

Ectotherms, including lizards, are widely used as a model system to address questions regarding the impact of thermal environment on the evolution of physiological traits (e.g., Bauwens *et al.* 1995; Angilletta 2009; Huey *et al.* 2009). Multiple lines of evidence suggest that thermal physiology of diverse lizard clades is highly conserved (i.e., the conservatism hypothesis; Bogert 1949; Hertz *et al.* 1983; Van Damme *et al.* 1990), leading to the prediction that lizard thermal physiology lacks evolutionary lability (reviewed in Hertz *et al.* 1983). Two mechanisms have been proposed to account for the apparent conservatism of lizard thermal physiology. First, behavior buffers thermal physiology from directional selection (Huey *et al.* 2003). Second, genetic constraints limit the ability of physiological traits to respond to environmental changes

(Van Damme *et al.* 1990; Huey *et al.* 2009; Sinervo *et al.* 2010). The conservatism of lizard thermal physiology has led to the prediction that lizards are particularly susceptible to rapid changes in climatic conditions (Huey *et al.* 2009; Sinervo *et al.* 2010). A recent survey of lizard populations across a vast geographic area partially supports this prediction by documenting local extinctions of species or populations over the last few decades (Sinervo *et al.* 2010). This problem is predicted to be most acute for tropical lizards, in which thermal conservatism is generally accompanied by a lack of phenotypic plasticity in response to thermal conditions (Tsuji 1988; Ghalambor *et al.* 2006; and references therein; Huey *et al.* 2009).

Over the past two decades, ecologists and evolutionary biologists have taken advantage of human-mediated species introductions to evaluate the responses of populations to novel environments over evolutionarily brief time intervals (e.g., Lee 1999; Huey *et al.* 2000; Herrel *et al.* 2008). The results of these studies are particularly robust when the age and putative source of the introduction is known, because this allows for direct comparisons between the introduced population and its source (e.g., Reznick *et al.* 1990; Losos *et al.* 1997; Kolbe *et al.* 2012). Here, we take advantage of the recent introduction (~35 years ago) of the tropical Caribbean lizard *Anolis cristatellus* to a sub-tropical region of North America, where winter temperatures are markedly colder than in the ancestral region.

Anolis cristatellus is native to the Greater Puerto Rican Bank, but established populations were first recorded in Miami, Florida, in 1975 (Schwartz & Thomas 1975). A phylogeographic study of *A. cristatellus* using mitochondrial DNA data demonstrates that the source of the Miami introduction used in this study was located within Las Cabezas de San Juan region, a continuous area of xeric forest located in northeastern Puerto Rico (Kolbe *et al.* 2007). Given the history of this introduction over the past 35 years, the source and introduced populations have experienced two distinct temperature regimes (Figure 6A). In particular, minimum air temperatures during the winter months can average 10°C cooler in Miami than in Puerto Rico. However, this pronounced difference in temperature is not present with regard to the maximum temperature experienced by the introduced and source populations. Therefore, this introduction provides a unique opportunity to evaluate the lability of thermal physiology over an ecological timescale.

The natural history of *A. cristatellus* is well known, making this species an excellent system with which to test predictions of the conservatism hypothesis. First, *A. cristatellus* has served as a model organism for studies of thermal ecology and physiology of anoles and tropical lizards in general (e.g., Hertz 1992a; Hertz 1992b; Hertz *et al.* 1993). Second, previous research has documented that, within the island of Puerto Rico, *A. cristatellus* populations occupying distinct thermal habitats do not differ in thermal physiology, particularly in preferred temperature (Hertz *et al.* 1993), critical

thermal maximum (CT_{\max} ; Huey & Webster 1976), and optimal sprint temperature (see Chapter 2; Gunderson & Leal 2012). Finally, *A. cristatellus* have served as an empirical example of how behavioral thermoregulation may buffer populations from experiencing divergent selection pressures when they inhabit different thermal regimes (Huey *et al.* 2003).

In this study, we ask whether thermal physiology, specifically cold tolerance (critical thermal minimum [CT_{\min}]) and heat tolerance (CT_{\max}) have diverged between the introduced and source populations of *A. cristatellus*. The conservatism hypothesis predicts that both populations should exhibit similar values of CT_{\min} and CT_{\max} . However, if traits are labile, we predict that CT_{\min} should have diverged between populations; specifically, we predict that individuals from Miami should tolerate colder temperatures than individuals from Puerto Rico. For the Miami population, we also evaluate whether CT_{\min} varies across seasons (early spring vs. fall), periods during which lizards experienced distinct thermal regimes. Because of the tropical evolutionary history of *A. cristatellus*, we predict that CT_{\min} should not vary across seasons (Janzen 1967; Ghalambor *et al.* 2006).

3.3 Methods

3.3.1 Study system

Anolis cristatellus is a medium-sized (males can reach 76 mm in snout-vent length), gray-brown, sexually dimorphic, arboreal lizard that is commonly found

perching on the lower portion of tree trunks. We collected *A. cristatellus* at two sites. On the island of Puerto Rico, lizards were collected at Las Cabezas de San Juan region (18° 16.204'N, 65° 37.845'W), located in northeastern Puerto Rico, at an elevation of 10 m. In Miami, Florida, lizards were collected in the vicinity of the village of Pinecrest (25° 40.167'N, 80° 17.138'W).

Lizards were brought to our laboratory and housed individually in plastic cages (29 cm x 21 cm x 21 cm) with a wooden dowel perch. Cages were located inside an environmental control chamber kept under a 12L:12D photoperiod cycle at a constant temperature of 28°C and 60% relative humidity. Lizards were watered daily and were fed crickets every other day.

3.3.2 Experimental design

The CT_{\min} and CT_{\max} are the lower and upper body temperature thresholds, respectively, at which a lizard loses the ability to right itself (Huey & Stevenson 1979). These metrics are often used as indicators of ecological death (i.e., physiological impairment, which would be lethal if maintained). We first explored whether the CT_{\min} of *A. cristatellus* from the Miami population varies across seasons. Lizards were collected in the vicinity of the village of Pinecrest during April 10–11, 2010 (early spring), and on October 1, 2010 (early fall). The CT_{\min} of lizards collected during the spring was measured during April 17–19, 2010, and the CT_{\min} of lizards collected during the fall was measured during October 22–23, 2010. With the exception of one female that was

measured as part of the collection of spring data, all other lizards used in this study were male. Lizards from Puerto Rico were not included in this experiment, because ambient temperatures at Las Cabezas de San Juan are relatively constant throughout the year (Figure 6A).

We next tested whether CT_{\min} has diverged between lizards from Puerto Rico and Miami. We collected lizards at Las Cabezas de San Juan on August 9, 2010, and measured their CT_{\min} during September 9–13, 2010, for comparison with the CT_{\min} of the Miami lizards that were collected in the fall. Individuals from both populations had experienced similar temperature regimes in their respective habitats for 4 weeks before collection (Figure 6B) and were maintained under identical laboratory conditions for 21 days before testing.

Finally, we tested for differences in the CT_{\max} of lizards from Miami and Puerto Rico. As was the case for the fall measurements of CT_{\min} , individuals were maintained under identical laboratory conditions for 21 days before data collection. Lizards from Miami were collected on December 23, 2011, and their CT_{\max} was measured on January 15, 2012. Lizards from Puerto Rico were captured February 4, 2012, and their CT_{\max} was measured February 26, 2012.

3.3.3 Measurement of critical thermal limits

To measure CT_{\min} , we placed the tip of a long thermocouple probe (~1.5 m, 40 gauge) inside the cloaca of a lizard and secured it to the base of the lizard tail with a

small piece of surgical tape. The probe was connected to an Omega digital thermocouple thermometer (model HH603A, type T, sensitivity 0.1°C), which allowed lizard body temperature to be monitored continuously throughout the experiment. Next, the lizard was loosely tethered to a piece of cardboard (29 cm x 21 cm) with a piece of dental floss looped around the waist, taking the precaution that limb movement was not affected. The cardboard was placed inside an incubator (Percival model I30NLC8) maintained at a temperature of 2°C . Lizards were removed from the incubator to assay righting ability at the following target body temperatures: 14.0, 13.0, 11.5, 10.0, 9.0, 8.0, and 7.0°C . Righting ability was evaluated by placing the lizard onto its back with tweezers and gently pinching the hind limbs with the tweezers to induce a righting response over a 20-s period. If the lizard righted itself, it was placed back inside the incubator to be tested at the next-lower target body temperature. If the lizard was unable to right itself, it was placed back inside the incubator until its body temperature decreased by 0.5°C and was then removed and assayed again. A lizard that failed to right itself this second time was left out of the incubator and allowed to slowly warm up at room temperature. As the lizard warmed up, we tested its righting ability at 0.5°C intervals. The CT_{\min} of a lizard was recorded as the lowest body temperature at which it righted itself during the cooling or warming phase of the experiment. The experiments followed approved ethical and institutional guidelines for animal care.

To measure CT_{\max} , we affixed a thermocouple probe inside the cloaca of lizards and tethered them to a piece of cardboard as described above for CT_{\min} . To increase their body temperature, the lizards were placed under a 150-W double-mirror incandescent light bulb. We tested each lizard for righting ability at 1°C intervals starting at 36°C.

3.3.4 Climatic data

We obtained air temperature data for Miami and Puerto Rico at two timescales: long-term temperature data (i.e., collected over the past 40 years) and short-term data (i.e., the thermal regime experienced by the lizards in the field for 28 days before collection). Long-term temperature data were compiled to explore the typical temperature extremes experienced by Miamian and Puerto Rican lizards since the approximate time of the introduction. These data were obtained from the Southwest Regional Climate Center at the University of North Carolina, Chapel Hill. Miami data were gathered from National Climatic Data Center (NCDC) weather station 085658 and include records for the period 1970–2010. Puerto Rico data were collected from NCDC station 668412, located at the Roosevelt Roads Naval Base, which is within Las Cabezas de San Juan region, and include records for the period 1970–2003 (records were unavailable for the years 1999–2000 and 2004–2010). From these data, we calculated the monthly mean extreme maximum air temperature and the monthly mean extreme minimum air temperature (Figure 6A).

We also obtained average daily temperatures for the 4 weeks (i.e., short-term timescale) preceding the day of lizard capture in Miami and Puerto Rico. These measurements provided an opportunity to evaluate the similarity of the thermal conditions at both sites immediately before individuals were captured. In the case of the Miami population, data were obtained for both the spring and fall collecting periods. These short-term data were compiled from the NCDC Global Summary of the Day online database (Miami, NCDC station 722020; Puerto Rico, NCDC station 998247).

3.3.5 Statistical analyses

Data on CT_{\min} and CT_{\max} were not normally distributed. After confirming homogeneity of variances with F-tests (all $P > .05$), we used nonparametric Wilcoxon rank-sum tests to test for differences in thermal tolerance. Short-term climatic data did not meet the assumption of homogeneity of variances, and data on minimum temperatures for Miami during the fall were not normally distributed. We tested for differences in short-term climatic data using the Welch's t-test for unequal variances on rank-transformed climatic data (see Ruxton 2006 for a detailed description of this approach). All tests were two-tailed at a $p = 0.05$. Analyses were conducted using the R statistical programming language (R Development Core Team 2012).

3.3 Results

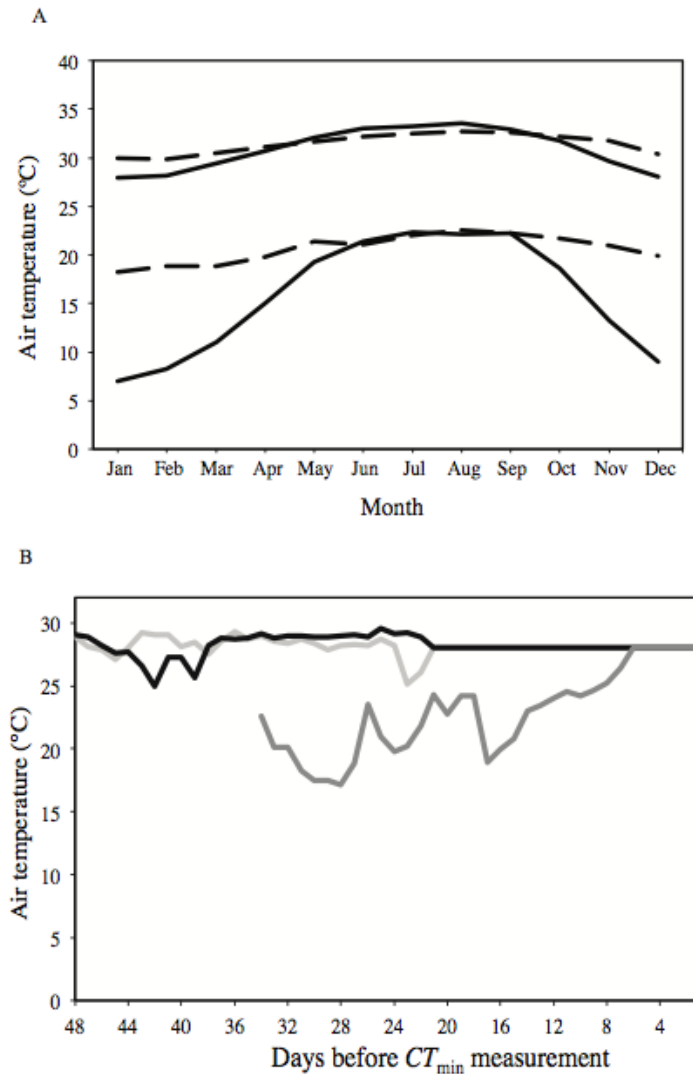


Figure 6. Ambient air temperatures experienced by individuals of *Anolis cristatellus* from the introduced and source populations. A, Average extreme air temperatures for each month over the past 40 years in Miami, Florida (solid lines) and “Las Cabezas de San Juan” region of Puerto Rico (dashed lines). The upper lines represent extreme maximum temperatures, whereas the lower lines represent the extreme minimum temperatures. B, Daily mean air temperatures experienced by individuals from Miami in the spring (dark gray line) and fall (light gray line) and Puerto Rico in the fall (black line) before critical thermal minimum (CT_{min}) measurements. The lines include the temperature experienced in the field 28 days before lizard collection as well as temperatures experienced in the laboratory.

Long-term climatic data demonstrate that, throughout the year, maximum air temperatures in Puerto Rico (i.e., Las Cabezas de San Juan region) and Miami have been very similar; however, minimum air temperatures have been considerably different (Figure 6A). The difference is most pronounced during the winter months (November–March), when average minimum air temperatures are of ten over 10°C cooler in Miami than in Puerto Rico (Figure 6A).

Heat tolerance, CT_{max} , did not differ between lizards from Miami and Puerto Rico (median CT_{max} for Miami lizards, 39.0°C, N = 11; median CT_{max} for Puerto Rico lizards, 39.0°C, N = 10; P = .970). However, lizards from Miami had significantly lower CT_{min} than lizards from Puerto Rico, which indicates that lizards from Miami have greater cold tolerance (Figure 7). The median CT_{min} of fall Miami lizards (10.0°C, N = 8) was 3.1°C lower than that of Puerto Rican lizards (13.1°C, N = 12; P < .001). The population difference in CT_{min} was observed despite the fact that, over a period of 28 days before lizard collection, mean daily air temperatures (\pm SD) did not differ significantly between sites (Miami, 28.2 \pm 0.9°C; Puerto Rico, 28.4 \pm 1.1°C; P = .120; Figure 6A), and mean daily minimum temperatures differed only by a magnitude of 1.8°C (Miami, 25.0 \pm 0.9°C; Puerto Rico, 26.8 \pm 1.9°C; P < .001).

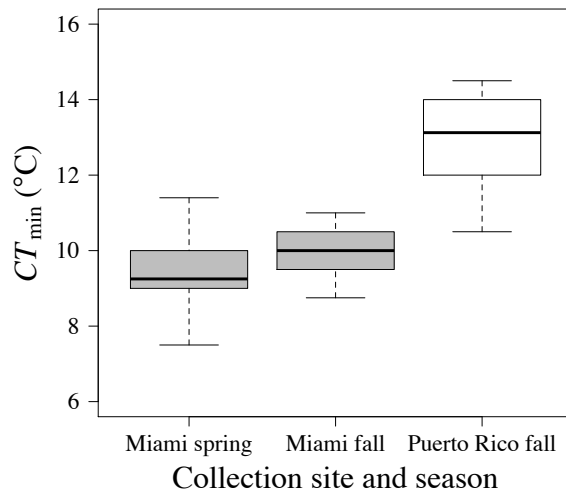


Figure 7. Box plots of the critical thermal minimum (CT_{min}) values of lizards from Miami and Puerto Rico measured in the spring and fall. Boxes encompass the second to third interquartile range, whiskers span the range of the data, and the thick bars indicate the median values.

Short-term field temperature data indicate that Miami lizards collected during the early spring experienced significantly colder temperatures than those collected during the early fall (Figure 6B). Spring lizards experienced a mean air temperature 6.5°C colder than that experienced by fall lizards (spring, $21.7 \pm 3.0^\circ\text{C}$; fall, $28.2 \pm 0.9^\circ\text{C}$; $P < .001$) and a mean minimum air temperature 8.4 C colder than that experienced by fall lizards (spring, $16.6 \pm 2.7^\circ\text{C}$; fall, $25.0 \pm 0.9^\circ\text{C}$; $P < .001$). Despite the difference in thermal regime, the CT_{min} of Miami lizards collected in early spring (9.3°C; N = 10) and early fall (10.0°C; N = 8) did not differ significantly ($P = .098$; Figure 7).

3.4 Discussion

Our results reject the hypothesis of thermal conservatism by demonstrating changes in the thermal physiology of a tropical lizard species over an ecological timescale (~35 years). The approximately 3°C differences in CT_{\min} between the introduced population and its source is in the direction predicted by the temperature regimes of each habitat, particularly with respect to the minimum air temperatures experienced during the winter (Figure 6A). This strongly suggests that the physiological differences can be adaptive, because increased cold tolerance could benefit lizards from Miami in a number of ways. Most obviously, greater cold tolerance should increase the probability of survival during cold winter temperatures. Other positive effects could be less conspicuous. For example, increased cold tolerance could increase the time available for activity during the winter, allowing more time for foraging and decreasing vulnerability to predation by limiting the amount of time that lizards are unable to move as a result of cold incapacitation.

As predicted, we found no difference between the CT_{\max} of lizards from Miami and that of lizards from Puerto Rico. Figure 6A shows that, over the period that *Anolis cristatellus* has been in Miami, the maximum ambient temperature experienced by the source and introduced populations is nearly identical. The observed values of CT_{\max} are nearly identical to those previously reported for multiple populations of *A. cristatellus* within Puerto Rico (Huey & Webster 1976).

Our results suggest that divergence in thermal tolerance (CT_{\min}) occurred rapidly (within ~35 generations) in a population of *A. cristatellus*. Faster rates of divergence in thermal physiology have been shown in other taxa, such as *Drosophila* and sticklebacks (e.g., Hoffmann *et al.* 2003; Barrett *et al.* 2011); however, given our knowledge of the thermal physiology of tropical lizards, which includes extensive work in *A. cristatellus* (Huey *et al.* 2003; Gunderson & Leal 2012; and references therein), our findings are unexpected (Huey *et al.* 2009; Sinervo *et al.* 2010). The predominant view across tropical lizards is that conservatism is the rule in thermal physiology, particularly over an ecological timescale.

Why the introduced *A. cristatellus* do not support the predictions of the conservatism hypothesis is an open question. The expectation of conservatism is based on the prediction that behavior can buffer thermal physiology from selection (reviewed in Huey *et al.* 2003) and that the temperature sensitivity of physiological traits lacks evolutionary lability, particularly over ecological timescales (e.g., Sinervo *et al.* 2010). Behavioral thermoregulation allows individuals to modify the selective environment (i.e., thermal regime) that they experience, thereby leading to a reduction or possible elimination of divergent selection pressure on thermal physiology (Bogert 1949), known as the “Bogert effect” (reviewed in Huey *et al.* 2003). However, the strength of the Bogert effect should be limited by the heterogeneity of the thermal environment. In Puerto Rico and Miami, *A. cristatellus* sleep on top of broad leaves exposed to the night air when

temperatures are lowest and when spatial thermal heterogeneity is at a minimum (Porter & Gates 1969; Huey *et al.* 1989). The long-term data for Miami (Figure 6A) show that ambient temperature can drop at least 3°C below the lizards' median CT_{\min} , which suggests that nighttime air temperatures can be a strong selective force. Our results suggest that, in situations in which behavioral thermoregulation cannot buffer selection on thermal physiology, tropical lizards may have the evolutionary lability to respond to new climatic conditions.

Adaptive phenotypic plasticity is an alternative mechanism that could account for the difference between the introduced and native populations of *A. cristatellus*, including seasonal acclimation in adults or irreversible developmental plasticity induced during embryonic or juvenile development (reviewed in Gienapp *et al.* 2008; Somero 2010). Seasonal acclimation has been demonstrated in other taxa, including temperate lizards (e.g., Wilson & Echternacht 1987; Tsuji 1988; Wilson & Echternacht 1990; Du *et al.* 2010). However, studies testing seasonal thermal acclimation in tropical anoles, including *A. cristatellus*, have found lack of support for this mechanism (Corn 1971; Rogowitz 1996b, a). Two additional lines of evidence suggest that acclimation does not account for the observed differences in CT_{\min} between the source and introduced populations. First, before collection, Miami lizards in the spring experienced a mean air temperature 6.5°C cooler and a mean minimum air temperature 8°C cooler than those experienced by fall lizards (see Section 3.3). Under acclimation, we would expect the

CT_{\min} of Miamian lizards to be lower in the spring than in fall (Tsuji 1988), but this pattern was not observed (Figure 7). Second, lizards from Miami and Puerto Rico experienced very similar environmental temperatures in the field for at least 28 days before collection (see the short-term temperature results) followed by identical laboratory conditions (i.e., 28°C air temperature) for approximately 21 days before measurement (Figure 6B). Under acclimation, we would expect the CT_{\min} of Miami and Puerto Rico lizards to converge after experiencing similar thermal regimes for 49 days; however, populations still exhibited significant differences in thermal tolerance (Figure 7).

Another possibility is that temperatures experienced during incubation or juvenile development have an effect on thermal physiology (Jensen *et al.* 2008; Angilletta 2009; Hofmann & Todgham 2010), although a recent synthesis of data for ectotherms suggests that adaptive developmental plasticity in response to thermal variation is rare (Angilletta 2009; Du *et al.* 2010). Extensive data on anole natural history suggest that it is unlikely that Miamian and Puerto Rican embryos will experience divergent thermal regimes. In Puerto Rico, *A. cristatellus* and other species in the cristatellus species group exhibit strong seasonality in breeding, which is primarily driven by changes in ambient temperature (Gorman & Licht 1974; Losos 2009). A strong seasonality in the breeding cycle has also been documented for *Anolis sagrei* in southern Florida, in which individuals are not reproductive from November through February (Lee *et al.* 1989).

Thus, *A. cristatellus* in Miami is unlikely to be reproductively active during the months when environmental temperatures differ substantially between Miami and Puerto Rico. Additional studies are needed to explore the possibility that incubation temperature can influence the CT_{\min} of *A. cristatellus*.

In summary, regardless of the mechanism, the adaptive lability of thermal tolerance in *A. cristatellus* over an ecological timescale is unexpected and provides a glimpse of hope for tropical lizards under the current conditions of rapid climate change. The relatively rapid divergence in CT_{\min} suggests the possibility that the physiological traits of tropical ectotherms are evolutionarily labile. However, their flexibility might be masked by the Bogert effect, which can compromise the accuracy of models predicting the evolutionary potential of thermal physiology. It should not be overlooked that it remains to be tested whether the lability of thermal tolerance is also present for the CT_{\max} (i.e., whether species or populations challenged with warmer temperatures would be able to respond to those challenges by increasing their upper thermal tolerance). At present, this remains an open question for most species of tropical ectotherms, particularly vertebrates. Human-mediated introductions or habitat disturbances in which populations experience warmer environments than those experienced by their source populations provide a unique opportunity to evaluate the lability of CT_{\max} over time scales relevant to predictions of global climate change (e.g., Angilletta *et al.* 2007). These systems are ripe for additional research, including gathering

much-needed empirical data to address the evolutionary potential of physiological parameters over short periods of time.

4. Re-thinking thermal constraints on ectotherm behavior: implications for understanding responses to climate change

4.1 Summary

Motile ectotherm populations are highly influenced by thermal constraints on activity. Thus, predicting the future activity budgets of ectotherm populations is crucial for estimating vulnerability to climate warming. Current activity models typically consider activity as a categorical variable with two states: active or inactive. In addition, activity is predicted to be constrained by the preferred temperature (T_p) of a population. Using field data collected on a tropical lizard and simulations parameterized by our observations, we evaluated three models of temperature-dependent activity with varying levels of thermal constraint. No models adequately predicted observed activity patterns. Lizards were active at body temperatures (T_b s) several degrees above and below the T_p range. Activity rates also demonstrated a graded response to temperature: activity was highest within the T_p range, but decreased gradually as T_b s moved above or below T_p . Additionally, the mismatch between current activity models and observed activity patterns increases as the mean T_b of a population rises. We propose a new model of thermal constraint on ectotherm activity that we term ACT (Activity as a Continuous Thermometer), and find that previously published data in other lizard species generally support it. The ACT model may represent a more biologically relevant framework for predicting the activity budgets of ectotherms under climate warming.

4.2 Introduction

Rising air temperatures associated with climate change are expected to result in an increase in ectotherm body temperatures (T_b s) on a global scale. Elevated T_b s can impact ectotherm ecology in myriad ways (reviewed in Angilletta 2009), often resulting in increased risk for extinction (Thomas *et al.* 2004; Parmesan 2006; Pounds *et al.* 2006; Sinervo *et al.* 2010; Bellard *et al.* 2012). Therefore, understanding how ectotherms cope with warmer temperatures, particularly under natural conditions, has become a central theme in ecology. Behavioral adjustments, such as shuttling between thermally suitable microhabitats, are one such suite of coping mechanisms for motile ectotherms (Bogert 1949; Kingsolver 1983b; Huey 1991; Huey *et al.* 2003; Ashton *et al.* 2009). Behavioral adjustments are also likely to be the first line of response of ectotherms to warming, because animals can typically respond more rapidly to changing environmental conditions via behavior than other biological processes (Huey *et al.* 2012).

One of the most important behavioral consequences of warming will be on the timing and intensity of activity that ectotherms can engage in (Porter *et al.* 1973; Grant & Dunham 1988; Dunham *et al.* 1989; Grant 1990; Christian & Weavers 1996) which we refer to as thermal constraints on activity. The vulnerability of many ectotherms to warming is predicted to be exacerbated by thermally-imposed reductions in activity time, as less time for activity translates into lower rates of growth and reproduction (Kearney *et al.* 2009; Sinervo *et al.* 2010; Sears *et al.* 2011; Buckley & Kingsolver 2012). For

example, a global analysis of lizard extinction risk under climate warming included thermal activity constraints by assuming that lizards are inactive when their body temperatures (T_b s) exceed their preferred temperature (T_p , the T_b organisms select given the choice in a thermal gradient)(Sinervo *et al.* 2010). They found that recently extinct lizard populations were those that had experienced warming that decreased the predicted daily activity window by approximately four hours during the breeding season. Based on these findings, they forecasts that up to 39% of lizard populations could go extinct by 2080 (Sinervo *et al.* 2010).

Despite the potential importance of thermal constraints on activity in mediating population responses to climate warming, our understanding of the impact of T_b on activity patterns is quite limited. Estimates of activity T_b s in the wild are common; however, these temperatures do not necessarily reflect the full range of T_b s that organisms will accept for activity. Many ectotherms may never approach their thermal limits for activity in nature, either because they live in thermally permissive environments (Deutsch *et al.* 2008; Huey *et al.* 2009; Gunderson & Leal 2012; Logan *et al.* 2013) and/or they reduce the variability in T_b s they experience via behavioral thermoregulation (Huey *et al.* 2003). In addition, most models of temperature-dependent activity treat activity coarsely, as a discrete variable with two possible states: active or inactive. For example, a classical activity model is shown in Figure 8A,B. In this model, ectotherms are assumed to be active only when their T_b is within the T_p range (Adolph &

Porter 1993). Such discrete activity models are frequently incorporated into analyses of the vulnerability of ectotherms to warming. However, activity rates may respond to thermal variation in a continuous manner, as do the physiological rate-processes that underlie activity (i.e., locomotion; Dell *et al.* 2011). To our knowledge, the consequences of considering activity as discrete vs. continuous with respect to thermal variation has not been explored.

Here, we evaluate the congruence between various activity models and observed activity patterns in *Anolis cristatellus*, a classic model system in behavioral and physiological thermal ecology (Huey 1974; Huey & Webster 1976; Hertz 1992a; Hertz 1992b; Hertz *et al.* 1993; Huey *et al.* 2003; Gunderson & Leal 2012; Leal & Gunderson 2012). The three models that we evaluate, from most to least thermal constraint, are 1) *T_p Only*: lizards are active only when their T_b is within the T_p range (Adolph & Porter 1993; Figure 8A,B), 2) *T_p and Lower*: lizards are active unless their T_b exceeds the upper bound of the T_p range (e.g., Sinervo *et al.* 2010 Figure 8C), and 3) *All T_p*: activity patterns are unrelated to T_p , and T_b does not influence activity rates while lizards are active (Figure 8D).

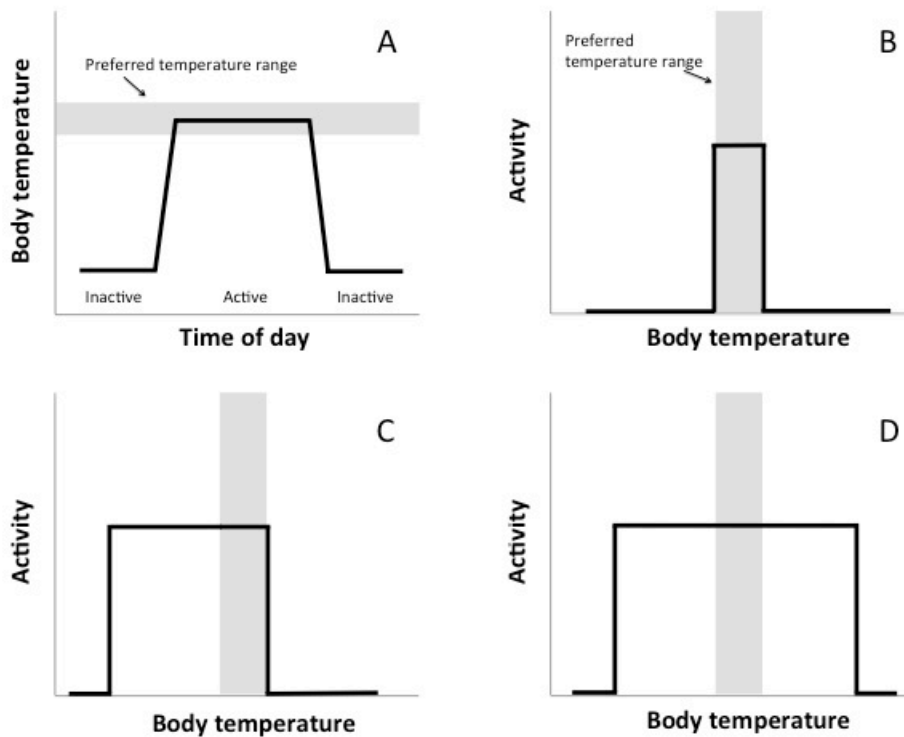


Figure 8. Models of temperature dependent activity. A) T_p Only, B) T_p Only represented along different axes C) T_p and Lower. D) All T_p .

In order to test these models, we collected data on the activity and T_{bs} of free ranging *A. cristatellus* during the peak of the breeding season in 2010 (Gorman & Licht 1974). Data were collected on two populations, one inhabiting a mesic forest and the other found in a xeric forest on the island of Puerto Rico. Activity was treated as a continuous variable, meaning the rate of activity was measured for each individual. We employed two separate approaches to assess the agreement between activity models and observed activity patterns of *A. cristatellus*. In the first approach, we generated an

activity data set for each activity model by transforming the observed data by the assumptions of each model. These new data sets were then compared to the observed data set. This provided an estimate of how the models perform under the thermal conditions we observed in 2010. In the second approach, we simulated activity rates of *A. cristatellus* with higher T_b s to explore whether the performance of activity models change under warmer conditions. Simulated data sets were constructed in which the lizards either adhered to the assumptions of the various activity models, or did not. For these simulations, expected activity rates were estimated based on the activity patterns we observed in our field study.

We found that none of the activity models tested accurately reflected the observed activity of *A. cristatellus* in all habitats or under all thermal conditions. Thus, based on our results, we propose a new model of thermal constraints on ectotherm activity which we term ACT (Activity as a Continuous Thermometer). This new model primarily differs from current activity models by predicting that activity does not occur only within the T_p range, and by describing activity as a continuous variable (a rate) that is sensitive to the T_b of the individual.

4.3 Methods

4.3.1 Study system

Anolis cristatellus is a small-to-medium sized grey-brown, sexually dimorphic, arboreal lizard typically found on the ground or on perches up to 2 m in height (Rand

1964). *Anolis cristatellus* is endemic to the islands of the Greater Puerto Rican Bank and was historically found from sea level to mid-elevation throughout the island of Puerto Rico, including both mesic and xeric habitats (Rand 1964; Heatwole 1976; Hertz 1992b).

4.3.2 Study system

We conducted focal observations on a total of $n = 299$ lizards. Observations were split between lizards occurring in two different habitat types: mesic ($n = 158$) and xeric ($n = 141$) forest. The mesic forest has a high, relatively closed canopy while the xeric forest is dominated by scrubby trees and has a more open canopy. In general Puerto Rican xeric forests receive less rainfall than mesic forests, by as much as 10 cm per month (Daly *et al.* 2003; Brandeis *et al.* 2009). Data collection in the mesic habitat occurred from July 26-August 4 2010 ($n_{\text{males}} = 81$; $n_{\text{females}} = 77$) at Bosque Estatal de Cambalache (18°26.998 N; 66°35.642 W; elevation 10 m), whereas data collection in the xeric habitat occurred from July 13-24 2010 ($n_{\text{males}} = 71$; $n_{\text{females}} = 70$) at Bosque Estatal de Guánica (17°58.246 N; 66°52.236 W, elevation 5 m). One lizard from Guánica was recorded as having an unusually high activity rate and T_b and was removed from analyses as an outlier. At both sites focal observations were conducted from 07:30 – 17:30 h each day, excluding periods of rain. Our target was to follow each focal lizard for 15 min, but lizards occasionally disappeared from view before an observation was finished. As a result, the mean duration of focal observation was 13.7 min (the minimum observation time was approximately 3 min) with a total of 68.4 h of observation.

Focal lizards were located by walking slowly through the forest along transect lines that followed randomly-generated compass directions and starting in a different haphazard location every day. This method ensured that no individual was observed more than once, and allowed us to sample large areas of each forest. Once spotted, focal lizards were observed at a distance of 3-4 m and their behaviors were recorded using a palm-pilot running Noldus Observer XT software (Noldus Information Technology, Inc., Leesburg, VA). For each individual we collected time-stamped activity data, which included agonistic encounters, feeding events, mating, and signaling displays (i.e., production of head-bobs, push-ups, and dewlap flashes; Jenssen 1977). Based on these data an activity rate was assigned to each lizard, calculated as the proportion of the total observation time that the lizard was active.

We estimated the T_b of each lizard by recording the temperature of a copper lizard model placed at the location where the lizard was perched for the majority of the observation. The copper models were constructed using a mold of *A. cristatellus* and have been used in several studies of *A. cristatellus* thermal ecology (see Hertz 1992a; Hertz 1992b for a detailed description of the models). The models are designed to have a low specific heat capacity, and thus their temperature rapidly reaches equilibrium under field conditions (Hertz 1992b). In a previous study at the same sites sampled here, we paired measurements of lizard T_b s with measurements of copper models placed at random locations near the lizards (Gunderson & Leal 2012). Copper model temperatures

and lizard T_b s were highly correlated when the model had the same basking status as the lizard (Pearson product-moment correlation, $R = 0.95$, $df = 137$, $P < 0.001$), as was the case in the present study. Therefore, the copper models provide a robust estimate of lizard T_b s in both habitat types under diverse conditions.

4.3.3 Statistical analyses

We tested for factors that significantly effect activity rates using a Type II sum of squares ANOVA model. Activity data were $\log + 0.01$ transformed to adhere to assumptions of normality. We began by including the following factors: habitat, sex, and T_p status (i.e., was the lizard within the T_p range or not) and all possible interactions. Non-significant terms were removed in a stepwise manner, beginning with interaction terms. None of the interactions were significant. Also, due to the unbalanced nature of our design, we constructed a series of models with the first-order terms entered in different orders to determine whether or not the significance of terms changed at $\alpha = 0.05$. We found that order did not affect the significance of any terms.

4.3.4 Evaluation of temperature-dependent activity models

We created three additional data sets, each one generated by transforming our observed activity data to meet the assumptions of one of the three activity models illustrated in (Figure 8): 1) *T_p Only*, which assumes that lizards are active only when their T_b is within their T_p range, 2) *T_p or lower*, which assumes lizards are active unless their T_b exceeds the upper bound of their T_p range, and 3) *All T_p* , which assumes that if

lizards are active their activity is not affected by their T_b . For T_p Only and T_p or Lower, we set a lizard's activity rate to 0 if the lizard's T_b was not within the prescribed activity bounds of the model. For All T_p , we assigned new activity rates to lizards who's T_b s were outside of the T_p range. For each of those cases we randomly drew their activity rate, with replacement, from the pool of activity rates of lizards that were within the T_p range. We estimated 95% confidence intervals for the grand mean activity rates of the observed and transformed data sets by implementing basic bootstrap resampling of each data set with replacement ($N = 1000$) using the "boot" package in the R statistical programming language V 2.15.0 (R Development Core Team 2012).

4.3.5 Simulations

To explore how activity models would perform under warmer conditions, we conducted individual-based simulations that combined our temperature-dependent activity data from 2010 with T_b data that we collected at the same two sites in the slightly warmer year of 2009 (Gunderson and Leal 2012). The mean T_b we measured in the mesic site, Bosque Estatal de Cambalache, in 2009 was very similar to that we measured in 2010 ($X_{2009} = 29.9^\circ\text{C}$, $X_{2010} = 28.8^\circ\text{C}$), as was the T_b range (T_b range 2009 = 26.4 - 31.1°C, T_b range 2010 = 25.7 C – 31.9°C). However, in the xeric site, Bosque Estatal de Guánica, the mean T_b was 2.2°C warmer in 2009 than in 2010 ($X_{2009} = 32.4$ C, $X_{2010} = 30.2$ C), although the range of T_b s measured was very similar (T_b range 2009 = 26.2 – 35.5°C; T_b range 2010 = 25.8 – 34.9°C)

In our simulations, lizards were tracked through an eleven-hour day broken into 44 fifteen-minute blocks. For each fifteen-minute block, a T_b was chosen at random from the pool of T_b s measured in 2009 during the appropriate time period, from the same habitat type. Next, the T_b was converted into an absolute deviation (d) from the T_p range, following (Gunderson & Leal 2012). Under this transformation, if a T_b falls within the T_p range, it has a d value of 0. If a T_b is 1°C from the T_p range (either above or below), it has a d value of 1, *etc.*

We binned our 2010 activity rate data according to the absolute d value of the focal lizard to create pools of activity rates from which to draw for our simulated lizards. The activity rates were binned as follows: $d = 0$; $0 < d \leq 1$; $1 < d < 2$; $d \geq 2$. For example, if a simulated lizard had a d value of 0 for a particular time block, its activity rate for that block was drawn from the pool of activity rates measured for lizards with a d of 0. If a simulated lizard had a d value of 1.5 for a particular block, its activity rate for that block was drawn from the pool of activity rates measured for lizards with a d greater than one but less than or equal to two.

4.4 Results

Individuals of *A. cristatellus* in both the mesic and xeric habitats were commonly active outside the of the T_p range (Figure 9). Focal lizards in the mesic habitat were outside

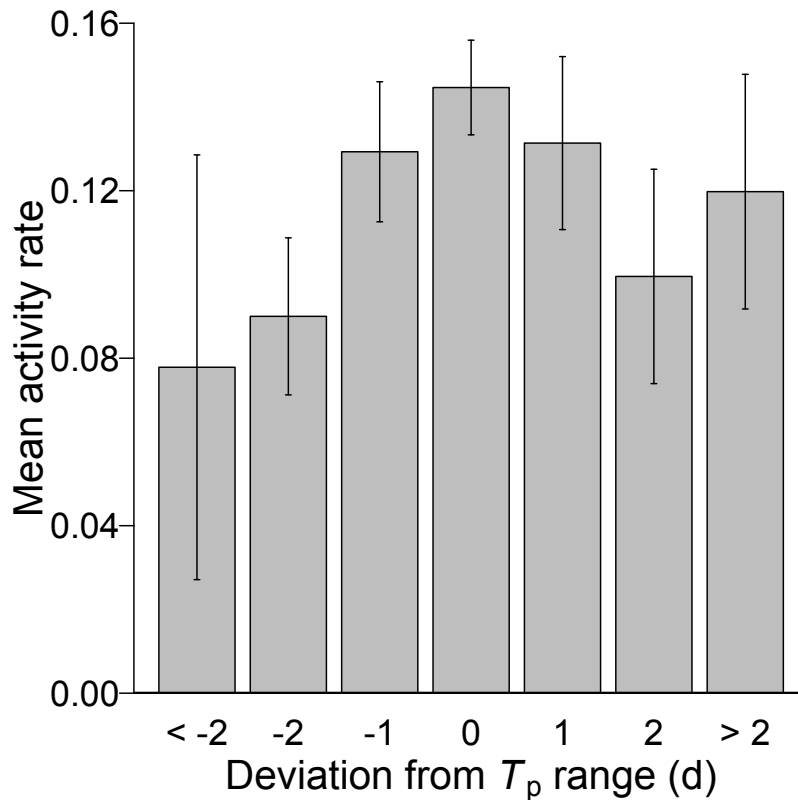


Figure 9. Mean activity rates of *A. cristatellus* binned by T_b scaled to d , the deviation from the T_p range. Data from focal lizards observed in the mesic and xeric habitats are combined. Error bars indicate \pm SE.

of the T_p range 42% (67/158) of the time, with most of those (62/67) below the T_p range. In the xeric habitat, focal lizards were outside of the T_p range 65% (92/141) of the time, and contrary to the mesic habitat lizards, most of those individuals (56/92) were above the T_p range. In both habitats, activities performed outside of the T_p range included locomotion, feeding events, social displays (e.g., head bobs, dewlap extensions) between males and females, and copulations (Figure 10).

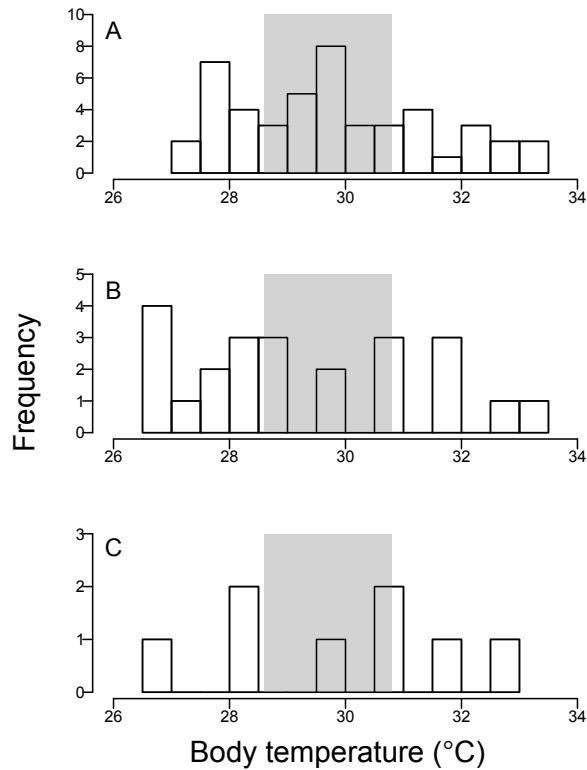


Figure 10. Frequency distributions of the T_b s of focal lizards that engaged in various categories of activity. A) Feeding, B) Male-female displays, C) Copulations. Grey bars indicate the T_p range.

Table 6. Summary of ANOVA results testing factors that affect activity rate in *Anolis cristatellus* on the island of Puerto Rico.

	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Habitat	1	2.524	2.327	0.128
Sex	1	4.32	3.983	0.047
Tp	1	4.711	4.343	0.038
Residuals	295	1.085		

Lizards from mesic and xeric habitats did not differ in their activity rates ($F_{1,295} = 2.327, P = 0.128$; see Table 6 for ANOVA model summary). However, females had lower activity rates than males ($F_{1,295} = 3.983, P = 0.047$) and individuals with T_b s within the T_p range had higher activity rates than those with T_b s outside the T_p range ($F_{1,295} = 4.343, P = 0.038$). Although lizards from the mesic and xeric habitats did not differ in mean activity rates, their daily patterns of activity exhibit differences that are consistent with the thermal regimes of their respective environments (see observed data, Figure 11A,B). In the more shaded mesic habitat lizards are usually within their T_p range during mid-day, and are often below the T_p range in the early morning and late afternoon (Gunderson & Leal 2012). Paralleling the changes in T_b throughout the day, lizards in the mesic habitat had their highest rates of activity during the middle of the day, and lower rates of activity early in the morning and late in the evening. Conversely, in the less shaded xeric habitat lizards are most often within their T_p range early in the morning and late in the afternoon, and are warmer than the T_p range during mid-day (Gunderson and Leal 2012). In the xeric habitat, lizards were most active in the morning and late afternoon, and have lower rates of activity during midday.

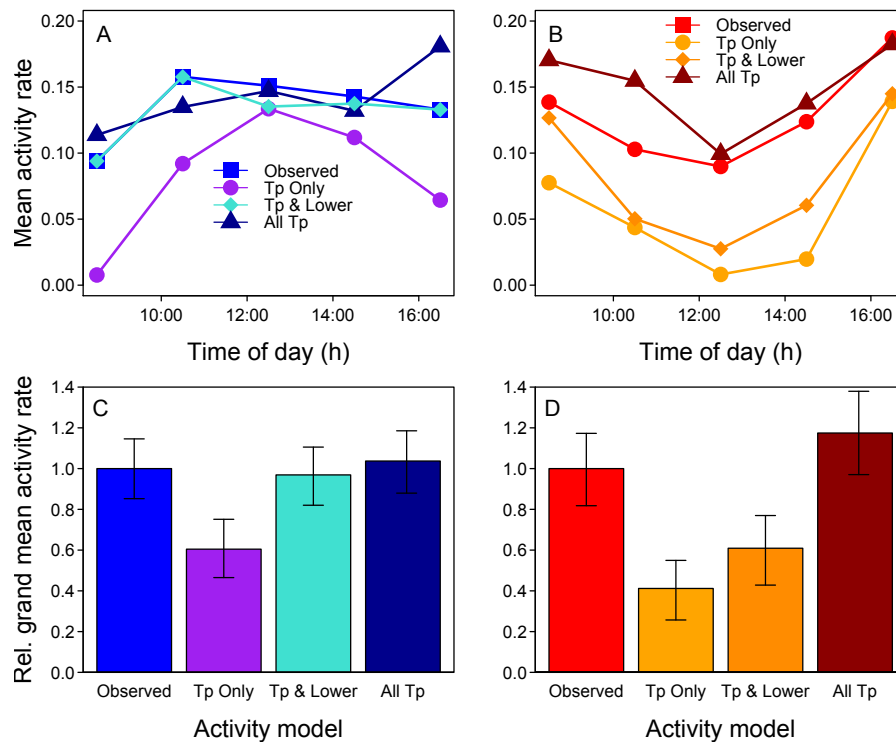


Figure 11. Summary of observed and model-transformed activity data for *A. cristatellus* in two habitat types: mesic forest (A, C) and xeric forest (B, D). Panels A, B: mean activity rate binned by time of day. Panels C, D: Grand mean activity rates of models relative to the observed grand activity rate (i.e., grand mean model activity rate/grand mean observed activity rate). Error bars indicate bootstrapped 95% confidence intervals.

How well do the various activity models predict the observed activity levels of *A. cristatellus*? In the mesic habitat, the activity levels predicted by the *T_p and Lower* and *All T_p* models were very similar to what we observed. Both models predicted grand mean activity rates within 4% of the measured grand mean (Figure 11A,C). In contrast, the *T_p Only* model underestimated the grand mean activity rate by 40% (Figure 11A,C).

In the xeric habitat, both the *T_p Only* and *T_p and Lower* models under-estimated observed rates, with grand means 59% and 39% lower than the measured value, respectively (Figure 11B,D). Conversely, the *All T_p* model over-estimated the grand mean activity rate in the xeric habitat by 17% (Figure 11B,D).

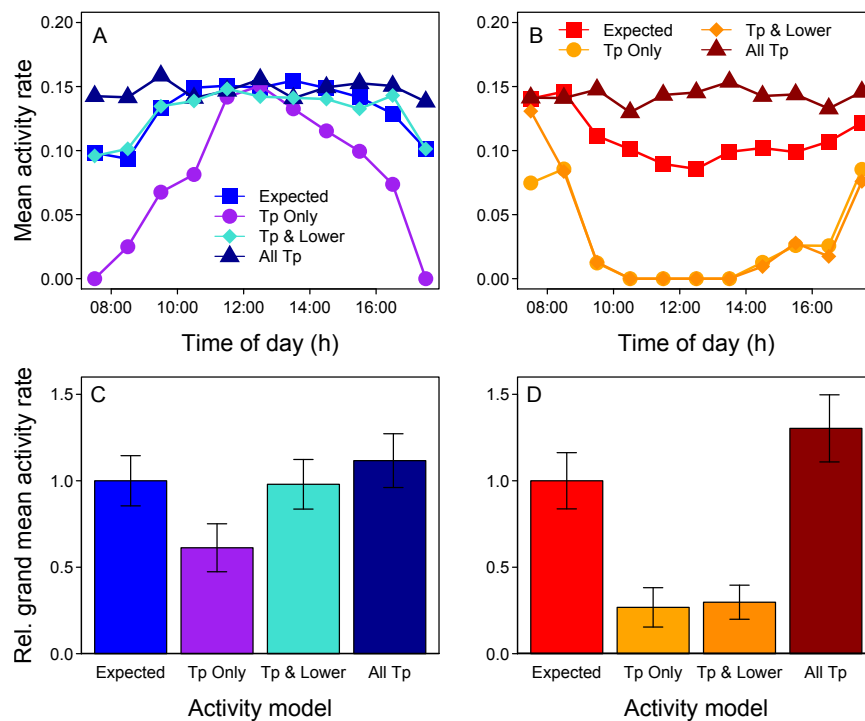


Figure 12. Summary of simulated activity data using 2009 T_b data for *A. cristatellus* from two habitat types: mesic forest (A, C) and xeric forest (B, D). Panels A, B: mean activity rate binned by time of day. Panels C, D: Grand mean activity rates of models relative to the expected grand mean activity rate (i.e., grand mean model activity rate/grand mean expected activity rate). Error bars indicate \pm SD.

How do the activity models perform under the slightly warmer T_b s we measured in 2009? According to our simulations, in the mesic habitat the *T_p and Lower* model underestimated the expected grand mean activity rate by only 2%, while the *All T_p* model overestimated the expected grand mean by 12% (Figure 12). In the mesic habitat the *T_p Only* model was the worst predictor of the expected result, underestimating the model result by 39% (Figure 12A,C). In the xeric habitat, the *T_p Only* and *T_p and Lower* models underestimated the expected grand mean activity rate by 73% and 70%, respectively, while the *All T_p* model overestimated the expected grand mean by 30% (Figure 12B,D).

4.5 Discussion

For decades thermal constraints on activity have been recognized as a central component of thermal ecology (Porter *et al.* 1973). Nonetheless, our understanding of thermal constraints on activity is surprisingly limited. We conducted a fine-scale analysis of the relationship between T_b and activity in free ranging *A. cristatellus*, and compared our results to those expected under various activity models. Our findings challenge the dominant view of how ectotherms behaviorally respond to thermal variation and demonstrate that considering activity at a fine scale can provide valuable insights into the effect of temperature on activity budgets. Our findings also have important implications for understanding the vulnerability of ectotherms to global climate change.

We found that individuals of *A. cristatellus* remain active several degrees above and below the T_p range (Figure 9, see also Gunderson & Leal 2012). In addition, lizards with T_b s above and below the T_p range engaged in the same activities as lizards within the T_p range, including feeding, social interactions (e.g., inter-sexual signaling displays) and mating (Figure 10). However, mean activity rates were highest within the T_p range (Table 6). This indicates that, although activity was not limited to the T_p range, attaining a T_b within the T_p range whenever possible is likely to result in an increase in activity levels.

We compared the fine-scale patterns of *A. cristatellus* activity that we measured under natural conditions with results expected under the following activity models: 1) *T_p Only*, 2) *T_p and Lower*, and 3) *All T_p* (Figure 8). The *T_p Only* model was a poor predictor of lizard activity patterns in both the mesic and xeric habitats (Figure 8A,B). This can be explained by the observation that *A. cristatellus* frequently remained active both above and below the T_p range. In addition, the maintenance of activity above the T_p range explains why the *T_p and Lower* model was a poor representation of lizard activity in the xeric habitat but not in the mesic habitat (Figure 11). In the xeric habitat 40% (56/141) of focal lizards were active above the T_p range, whereas in the mesic habitat only 3% (5/158) of lizards were active above the T_p range. The *All T_p* model was the only model that performed reasonably well in both habitats, overestimating grand mean activity rates by 4% and 17% in the mesic and xeric habitats, respectively (Figure 11).

When we simulated the activity of *A. cristatellus* under the warmer T_{bs} that we measured in 2009, the results were qualitatively similar to the transformed results from 2010 (Figure 12). The T_p *Only* model was a poor predictor of the expected result in both habitats, and the T_p *and Lower* model was a good predictor of the expected result in the mesic but not the xeric habitat. In addition, the *All* T_p model performed well in the mesic habitat but less so in the xeric habitat (Figure 12). Despite the qualitative similarity, the activity models performed quantitatively worse in the xeric habitat simulations: the simulated T_p *Only* and T_p *and Lower* models underestimated the expected mean activity rate by over 70% (Figure 12B,D), while the *All* T_p model over-estimated the expected mean activity rate by over 30% (Figure 12B,D). The combined results of the empirical data and the simulations suggest that the discrepancy between current activity models and observed patterns of activity may be exacerbated as T_{bs} rise. This is a troubling finding given our need to accurately predict how organisms will respond to increasing T_{bs} due to climate warming.

In general, we found that models were either too constraining on activity (i.e., the T_p *Only* model in the mesic and xeric habitat, the T_p *and Lower* model in the xeric habitat) or overestimated mean activity rates (i.e., T_p *All* in the xeric habitat, particularly based on simulated 2009 results). The lack of congruence between our results and current activity models leads us to propose a new general model of temperature-dependent ectotherm activity (Figure 13), which we call the ACT (Activity as a

Continuous Thermometer) model. Two features distinguish the ACT model from those we have discussed. First, it predicts that activity can occur at T_b s several degrees above and below the T_p range. Second, it predicts that mean activity rates change in a graded manner as T_b changes (i.e., activity is not simply “on” or “off”). Specifically, we posit that mean activity rates increase in an approximately linear fashion with T_b up to a maximum within the T_p range, and then decrease approximately linearly again as T_b s increases beyond the T_p range. This feature of the model predicts that temperature does not impact activity budgets solely by constraining the duration of activity: it also has an effect on the intensity of activity within the activity window.

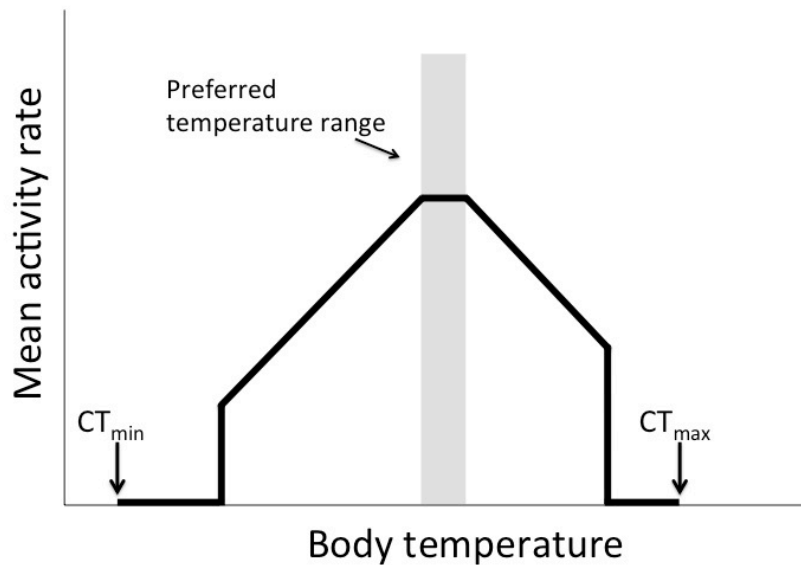


Figure 13. A graphical representation of the ACT (Activity as a Continuous Thermometer) model of thermal constraints on ectotherm activity.

The ACT model also posits that the relationship between T_b and activity is asymmetrical: the thermal limit for activity is reached more quickly as T_b s increase above the T_p range than when T_b s decrease below it. Although we did not observe the thermal activity limits of *A. cristatellus*, we base this prediction on the fact that for most ectotherms, including *A. cristatellus*, T_{pS} are closer to the upper critical thermal limit than they are to the lower critical thermal limit (Martin & Huey 2008). However, though we posit an asymmetrical relationship, we do not predict that the temperature-dependence of activity mirrors the temperature-dependence of physiological traits (i.e., thermal performance curves). This follows from the fact that we predict mean activity rates to be highest within the T_p range, whereas ectotherm T_{pS} are usually below the optimal temperature for performance (Martin & Huey 2008; see also discussion in Asbury & Angilletta 2010).

The ACT model is a general graphical hypothesis for the form of temperature-dependent activity in ectotherms. In its current form the ACT model does not predict how quickly activity rates will change as T_b s move away from the T_p range, or how much variation is expected around the mean activity rate at a particular T_b for a given population. As with most behavioral or physiological processes, parameter values will almost certainly vary among taxa, and estimating them will require the collection of fine-scale behavioral data that are currently rare.

The next step is to determine whether or not features of the ACT model are supported in other species. We are aware of only a handful of studies that have simultaneously collected data on both T_b s and activity rates in motile ectotherms. Nonetheless, those studies that do measure both traits observe behavioral patterns that are congruent with the general predictions of the ACT model. For example, graded behavioral responses to temperature have been observed under laboratory conditions in red-sided garter snakes (*Thamnophis sirtalis parietalis*) with respect to courtship displays (Hawley & Aleksiuik 1975) and in feeding rates of cabbage white caterpillars (*Pieris rapae*; Kingsolver 2000).

Even fewer studies have collected fine-scale data on activity rates and T_b s under natural conditions. Perhaps the most detailed study of this kind was conducted by Christian and Weavers (1996) on four species of Varanid lizard from Australia. They used radio-telemetry to measure T_b s and activity rates of individuals of each species in the field, and coupled those data with laboratory measurements of the same species' T_p ranges. To determine whether the patterns of activity observed for the four Varanids support the ACT model, we extracted data on mean activity rate and mean T_b for each species during the breeding season from the figures of Christian and Weavers (1996), using the free online software PlotDigitizer. We then tested for a correlation between these two factors (after converting T_b to d) using the Spearman rank correlation test. Mean T_b s rarely exceeded T_p s, and thus we were unable to test for congruence with the

ACT model above the T_p range. Nonetheless, in three of the four species activity rate correlated positively with T_b ($P < 0.05$), matching the predictions of the ACT model (Figure 14).

Several studies have documented that free-ranging ectotherms remain active even when their T_b s exceed the T_p range. For example, during summer months the Australian Agamid lizard *Pogona barbatus* is active at T_b s significantly higher than its T_p range, with maximal activity T_b s approximately 3°C warmer than the upper T_p boundary (Schäuble & Grigg 1998). In three species of Australian skink (genus *Carlia*), over 50% of individuals active at midday have T_b s above the T_p range in both the summer and winter (Vickers *et al.* 2011). Similarly, active individuals of the North American desert iguana (*Dipsosaurus dorsalis*) attain T_b s that are on average 4°C warmer than the mean T_p during the warmest times of the day during the summer (DeWitt 1967). In a similar vein, juvenile steelhead trout (*Oncorhynchus mykiss*) have been shown to remain active and feed at T_b s higher than their T_p (Spina 2007).

The ACT model has important implications for understanding the current thermal ecology of motile ectothermic species and for assessing the vulnerability of ectotherms to climate warming. Demographic parameters such as growth and reproductive rates are heavily influenced by the activity budgets of populations (i.e., Dunham *et al.* 1989; Adolph & Porter 1993). As a result, niche models that forecast the impacts of climate warming on ectotherms often incorporate activity budgets into their

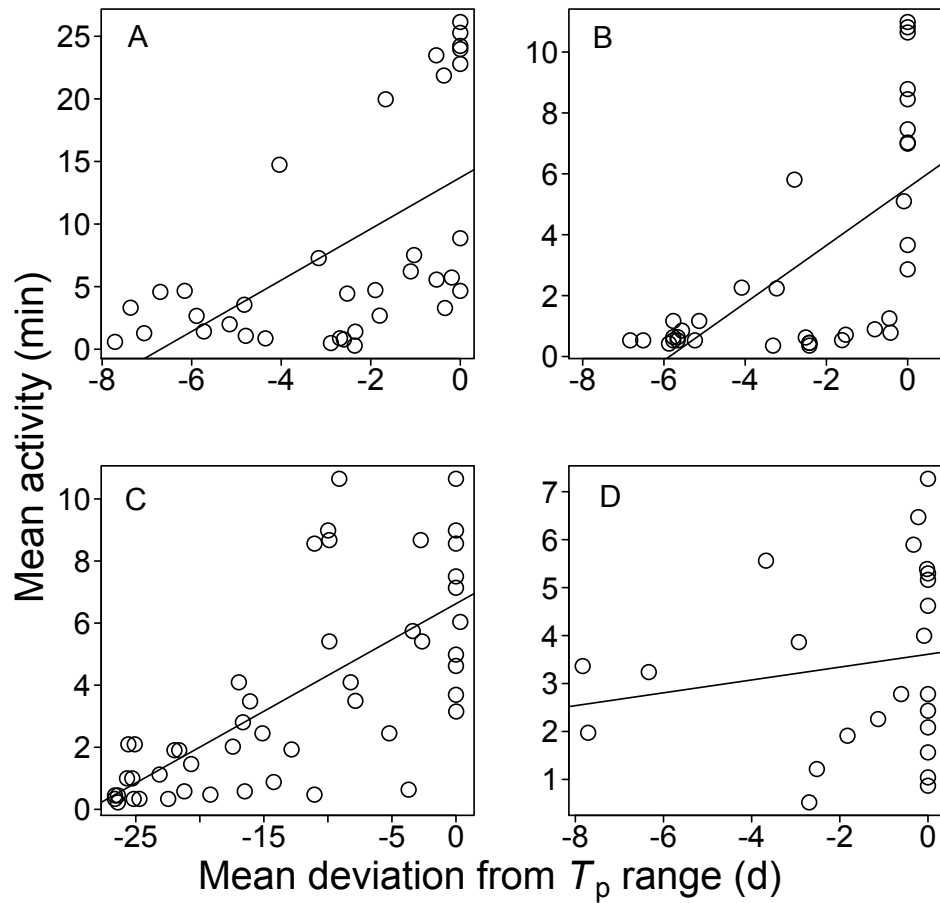


Figure 14. Mean activity rates vs. mean T_b (scaled to mean deviation from the T_p range, d) for four species of Australian Varanid lizards measured by Christian and Weaver (1996) during the breeding season. A) *Varanus panoptes*. B) *V. gouldii*. C) *V. mertensi*. D) *V. rosenbergi*.

analyses, and typically due so by assuming activity is constrained to occur within some estimate of the T_p range based on measurements of T_b s in the field or in laboratory thermal gradients (e.g., Buckley 2008; Kearney *et al.* 2009; Buckley *et al.* 2010; Sinervo *et al.* 2010). Our results indicate that assuming activity only occurs within the T_p range is

inaccurate and has the potential to greatly underestimate the activity budgets of ectotherms under current and especially future conditions. We also find that activity rates are unlikely to be the same at all temperatures that are permissive for activity. Thus, calculating realistic activity budgets under future thermal conditions likely requires weighting the amount of time that activity can occur by the distribution of expected T_{bs} . The ACT model also has implications for estimating the indirect effects of climate warming via species interactions. For example, encounter rates between interacting species such as competitors and predators may be influenced by changes in the intensity of activity as T_{bs} rise (Gilman *et al.* 2010; Vucic-Pestic *et al.* 2011; Dell *et al.* 2013).

Forecasts of biological responses to climate change based on species traits can only be as robust as our estimates of those traits (Buckley *et al.* 2010; Kearney *et al.* 2010; Buckley *et al.* 2011). Empirical studies of thermal constraints on activity should be a focus of future work, as our current understanding of the temperature-dependence of activity is extremely limited. Our model is potentially an important step toward filling this gap, facilitating robust predictions about how individuals, populations, and ultimately communities of interacting species will be affected by climate warming.

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Biography

Alexander Rahn Gunderson was born in Virginia, MN, USA, on April 28 1981. He received a Bachelor of Science degree in Biology, Magna Cum Laude, from Minnesota State University, Mankato in 2004. He then received a Master of Science degree from The College of William and Mary in 2007, and was awarded the Distinguished Master's Thesis in the Natural or Computational Sciences prize by The College of William and Mary Graduate School. At William and Mary he published "Resistance of melanized feathers to bacterial degradation: Is it really so black and white?" and "Evidence plumage bacteria influence plumage coloration and body condition of a passerine" in *The Journal of Avian Biology* and "Feather-degrading bacteria: A new frontier in avian and host-parasite research?" and "Reproductive success of eastern bluebirds (*Sialia sialis*) on suburban golf courses" in *The Auk*. Alex joined the laboratory of Dr. Manuel Leal at Duke University in 2007 and received the degree of Doctor of Philosophy in Biology in 2013 along with a Certificate in College Teaching from the Duke Graduate School. During that time he published "Tests of the contribution of acclimation to geographic variation in water loss rates of the West Indian lizard *Anolis cristatellus*" in *The Journal of Comparative Physiology B*, "Geographic variation in vulnerability to climate warming in a tropical Caribbean lizard" in *Functional Ecology*, and "Rapid change in the thermal tolerance of a tropical lizard" in *The American Naturalist*. From work he completed prior to graduate school, he has also published

“Early social experience patterns maltreatment of young birds” in *The Auk* and
“Complex facilitation and competition in a temperate grassland: loss of plant diversity
and elevated CO₂ have divergent and opposite effects on oak establishment” in
Oecologia. During his doctoral work, Alex received the Duke Biology Department
Graduate Research Grant, The Duke Center for Latin American and Caribbean Studies
Caribbean Travel Grant, the Duke Graduate School Student Travel Grant, the Sigma Xi
Sally Hughes-Schrader Travel Grant, the North Carolina Academy of Sciences Bryden
Student Grant, the Animal Behavior Society Student Research Grant, the Society for
Integrative and Comparative Biology Graduate Research Grant, a National Science
Foundation Doctoral Dissertation Improvement Grant, and the Duke University
Katherine Goodman Stern Fellowship. Alex is a member of the Society for Integrative
and Comparative Biology, the Animal Behavior Society, the American Society of
Naturalists, and the Society for the Study of Evolution.